17. *The Enteropneusta from Inyack Island, Delagoa Bay.*—By C. J. VAN DER HORST, University of the Witwatersrand, Johannesburg.
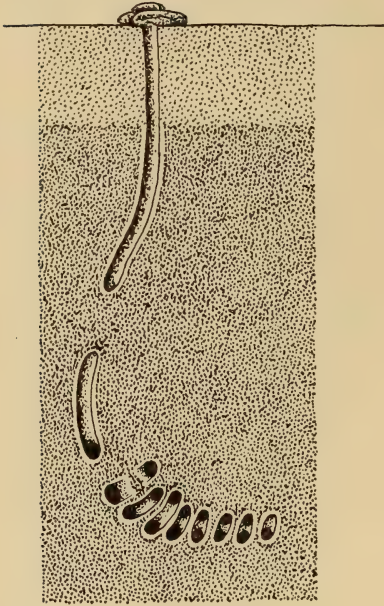
(With 70 Text-figures.)

CONTENTS.

THE island of Inyack, in Delagoa Bay, Portuguese East Africa, is an ideal place for collecting marine animals that live burrowing in the soil in the tidal zone. The eastern side of the island is exposed to the Indian Ocean, but along its northern, western, and southern sides it faces the Bay, which is here very shallow. As the difference between high and low tides can be over 3 metres, large flats and sand-banks are exposed at low tide. In the more sheltered bays these flats have a muddy soil; in places where a coral reef extends in front of the flats the mud is mixed with coral debris, but in the more exposed parts, where either the tidal stream or the action of the waves is stronger, large banks of pure sand occur. This is a place where one can expect Enteropneusta, and they are found here in great abundance. During the last few years I have visited Inyack repeatedly and have collected a great number of Enteropneusta. Up till now I found six different species, only one of which had previously been described. Another species was recently described by my student, Mrs. Kapelus, and in the following pages the description of the other four species is presented.

SACCOGLOSSUS INHACENSIS Kapelus.

The small enteropneust, *Saccoglossus inhacensis*, described by Mrs. Kapelus, occurs in countless numbers on the muddy flats west of the island. In nearly every spadeful of sandy mud one can find one or more specimens.

In a letter to "Nature" I described the characteristic burrow made by this animal in the mud; and now, after some further observations,

I am able to supplement the previous remarks (fig. 1).   At the place where the animal occurs most abundantly the black mud is covered by a layer, about 1 cm. in thickness, of yellow sand, and as this sand does not hold together so well as the mud, I was at first unable to detect the tube of the animal in the sand.   But later I found it extending from the surface to a depth of 4 to 7 cm.   The upper part of the burrow is irregularly coiled, but deeper down it takes the form of a regular spiral, consisting of up to eight turns.   The direction of the main axis of the spiral is variable; in the majority it was found to be approximately vertical, but it may even be horizontal.   In the dark mud this burrow is very conspicuous because it is lined by a thin layer of clear sand. This in turn is covered by a layer of slime, giving the inner surface a smooth and shiny appearance. As the sand is also cemented together by the slime the spirals are rather persistent; at least as many were found that had been vacated by the animals as inhabited ones.

FIG. 1.—Burrow of *Saccoglossus inhacensis* Kapelus.   Nat. size.

In the letter to "Nature" I stated that no indication of the presence of the animal was found on the surface during low tide.   Subsequently I found that this statement was wrong.   Like other enteropneusts, *Saccoglossus inhacensis* also makes heaps of coiled castings round the entrance of its burrow.   But these heaps are very small, and the coils seem to be not so persistent as in *Balanoglossus*.

Only when there is no disturbance at all the heaps, which are about 1 cm. wide and 2 mm. high, will preserve their typical form and then they cover the surface in great numbers at low tide.   In order to make these heaps of castings, the animals must protrude their hind-ends, where the anus is, above the surface of the sand.   Nevertheless, when one finds the animals in their burrow by digging, they invariably take up a position with the proboscis and anterior end of the body in

the irregularly formed upper part of the burrow, and the abdominal region is found in the spiral. Therefore the animal must be able to turn round in its burrow.

In miocene strata from different localities (Switzerland, Bavaria, Maryland, California, Mexico) fossilised internal casts in the form of spirals have been described under the name of *Xenohelix.* Several suggestions have been made about the origin of these spirals. Mansfield was of the opinion that they owe their origin to some marine plant; Heer supposes them to be the burrows of the mussel *Mactrina.* Abel, in his book "Vorzeitliche Lebensspuren," expresses the opinion that these spirals are the burrows of small crabs. Along tropical shores a small crab, *Mictyris,** may occur in countless numbers, and at the approach of danger these crabs disappear in the mud by digging a burrow in the form of a spiral. However, these burrows can be only of a very temporary nature, and as Abel wrote me later he has given up this opinion. The stratum in which these spirals are found was formed in a shallow sea with a very level shore, where mangroves grew in great abundance. It is exactly in such a place that the spirals of *Saccoglossus inhacensis* are found, and as these spirals are rather persistent, even when they are uninhabited, I venture to suggest that *Xenohelix* is the fossilised burrow of an enteropneust.

WILLEYIA DELAGOENSIS n. sp.

In November 1935, during a short stay on Inyack Island, I secured a single specimen of a pure white enteropneust on the flats at the west side of the island. It proved to belong to the genus *Willeyia,* established by Punnett for an enteropneust from Zanzibar. This genus is more or less intermediate between *Glandiceps* and *Spengelia;* it agrees with *Spengelia* in having peripharyngeal cavities and with *Glandiceps* in the absence of synapticula. As Punnett's description is not as extensive and accurate as it might have been, Spengel has expressed his doubts about the validity of the genus. However, I can corroborate Punnett's observations by the study of this specimen, and this leaves me in no doubt regarding the validity of the genus *Willeyia.* In addition to some minor characters, the only specimen that was collected at Inyack differs in one important feature from *Willeyia bisulcata* Punnett, *i.e.* in the extension of the gonads into the branchial region, a character which Punnett thought might be

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* *Mictyris* has not been recorded from South African shores, but the closely allied *Dotilla* is very common.—ED.

of generic importance. It is for this reason that I am describing this specimen as belonging to a new species of the genus *Willeyia*.

External Features.

In its external appearance *W. delagoensis* closely agrees with *W. bisulcata* according to Punnett's description (fig. 2). The proboscis is very elongated; in the fixed animal it has a length of 15 mm.
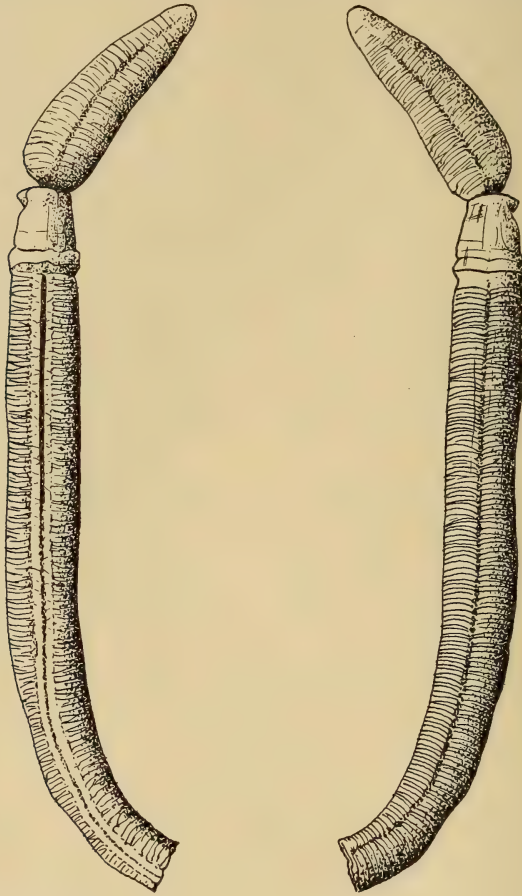

Fig. 2.—*Willeyia delagoensis* n. sp.   Dorsal (left) and ventral (right) views.   × 2.

and its greatest width is 5 mm.; in the living animal the proboscis was considerably longer. It also shows the longitudinal grooves along the dorsal and ventral mid-lines. The collar is about 5 mm. long

and is broader behind than in front. The proboscis is flattened, but the collar and the trunk, at least as far as the latter is present, are nearly cylindrical. There is no trace of genital wings or folds, not even behind the branchial region.

It is difficult to determine the length of the branchial region owing to the fact that the minute branchial pores open into a narrow and often deep groove, which fades away gradually in the anterior part of the genital region. By counting the sections it was possible to estimate that the branchial region is about 3 cm. long. The specimen was broken off in the genital region, so that it remains uncertain whether *Willeyia* has liver saccules or not. Punnett's specimen was also incomplete.

The animal, when alive, was of a pure white colour throughout.

Internal Anatomy.

Proboscis.—The epidermis of the proboscis consists of elongated cells. I can confirm Punnett's statement that the epidermis is almost destitute of glands, particularly as special glandular cells seem to be absent. A great number of the ordinary cells are swollen in their peripheral part, exhibiting the features of slime cells. It seems likely that all epidermal cells can secrete slime. As in *W. bisulcata*, elongated nuclei are arranged in a distinct row in the middle of the cells, and another layer of round nuclei is found in the basal part of the epidermis; the latter are evidently the nuclei of the nerve cells.

In *W. bisulcata* the circular muscle layer is about half as thick as the nerve layer; in *W. delagoensis*, however, these layers are of about the same thickness, except in the grooves where the nerve-fibre layer is better developed (fig. 3). Sensory cells could not be found in the epidermis of these grooves.

The muscle fibres are neither arranged radially nor in concentric layers; they are only more crowded in the central part of the proboscis than near the epidermis. It is well known that these longitudinal muscle fibres are attached by both ends to the basal membrane of the epidermis. Whereas the anterior end of each fibre pierces the circular muscle and attaches itself to the basal membrane independent of other fibres, one finds that in the posterior part of the proboscis the longitudinal muscle fibres collect in bundles, where they pierce the circular muscle layer and are attached to the basal membrane. In cross-section it seems therefore that the circular muscle layer is

interrupted; in reality this muscle layer will have the appearance of a network. The basal membrane is produced into an inwardly directed point or crest where the longitudinal muscle fibres are attached to it (fig. 4).

The proboscis coelomic cavity extends nearly to the top of the proboscis. Over its whole length it is separated into a right and a left part by a kind of dorso-ventral septum formed of connective tissue and muscle fibres (fig. 3). Near the anterior end of the glomerulus and again just in front of it there are a few openings in this septum by means of which the right and the left coelomic cavities intercommunicate. Punnett figures the right and the left coelomic parts as of equal size in *W. bisulcata*, but in *W. delagoensis* the right part is wider than the left, though taken together the two parts form a symmetrical figure; the septum between the two has evidently shifted towards the left side (fig. 3). Owing to the fact that this septum consists of fibres only and not of a limiting membrane, this shifting does not seem to be of great importance and the difference in size of the coelomic parts may not be of specific value.

The dorso-ventral muscle fibres go by way of this septum from the dorsal to the ventral mid-line, so that they deviate somewhat from the straight course. These fibres are thicker than those of the longitudinal or circular musculature. They do not form a continuous layer as they run separately; there may be one or more fibres visible in a cross-section or none at all. At the dorsal side of the septum the fibres are arranged in two longitudinal rows. Ventrally there is usually one row, but if there are two rows these lie very close together, except in the posterior part of the proboscis, just i . ont of the glomerulus, where the two rows are more distant from each other and can be easily discerned at the ventral side of the septum.
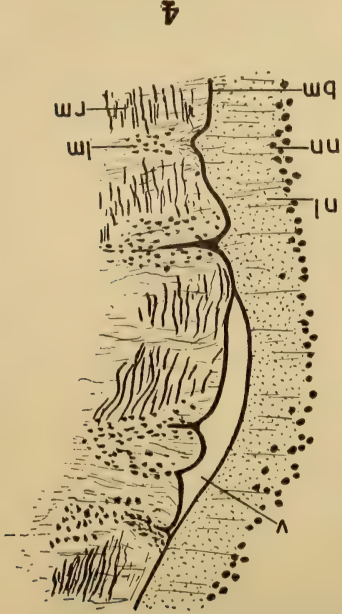
From the anterior to the caudal part of the proboscis the coelomic cavity gradually increases in width. This cavity is surrounded by a distinct epithelium which, as Punnett also mentions, is considerably thickened on the septum. It is somewhat lower in the right and the left angles of the cavities and it is very low along the dorsal and ventral border, although here also it is clearly visible (fig. 3). This epithelium does not seem to be of an excretory nature, as may be the case in some other Enteropneusta.

The ventral proboscis septum, as in *Glandiceps*, reaches to the top of the stomochord but leaves the vermiform process free. Its attachment to the basal membrane of the epidermis, however, does not reach so far forward, as a result of which the free edge of the
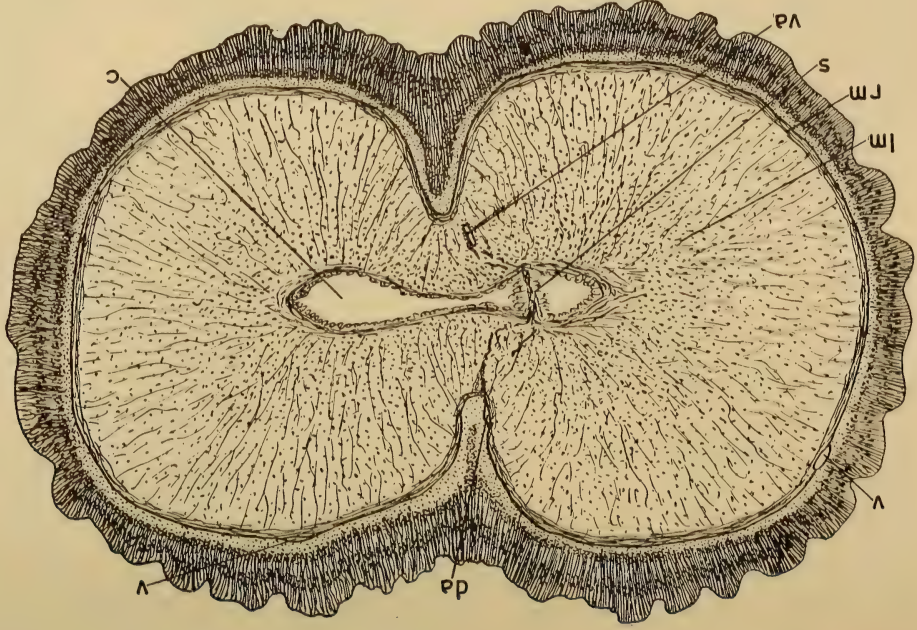
Figs. 3, 4.—*Willeyia delagoensis* n. sp. 3. Cross-section of proboscis in front of the central organs. ×34. 4. Part of a cross-section of the proboscis. ×215.

bm, basal membrane of epidermis. *c*, coelomic cavity. *da*, dorsal artery. *lm*, longitudinal musculature. *nl*, nerve-fibre layer. *nn*, nuclei of nerve cells. *rm*, ring musculature. *s*, septum. *v*, proboscis veins. *va*, ventral artery.

septum runs from the top of the stomochord to the epidermis in a
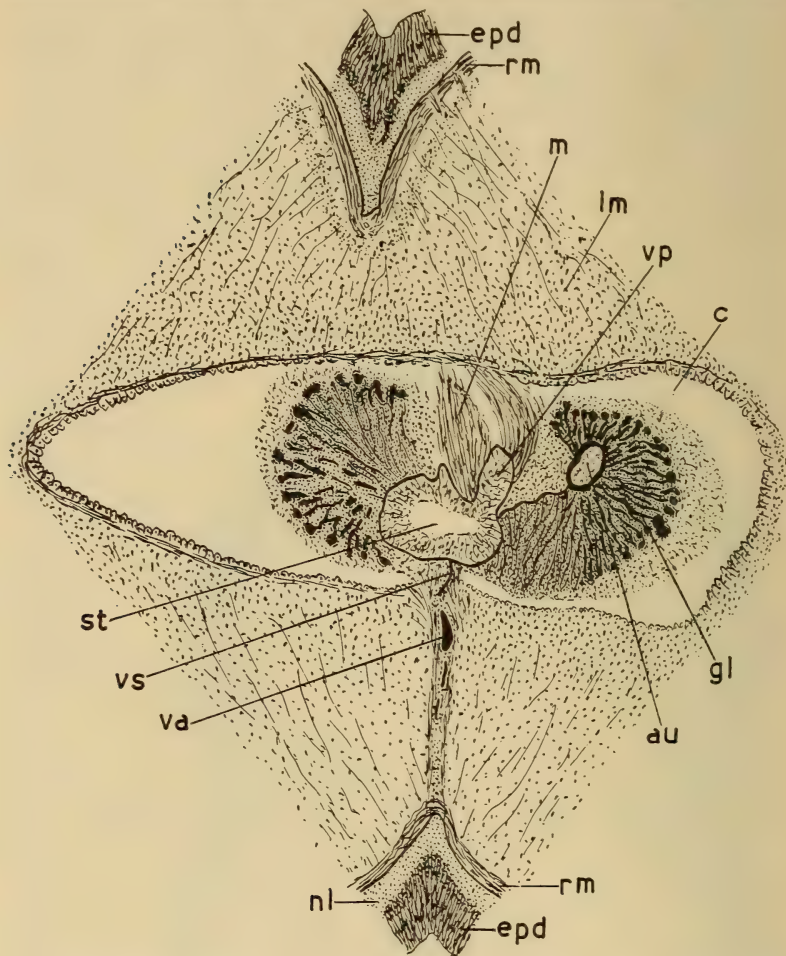ventro-caudal direction.   Probably on account of this the ventral

FIG. 5.—*Willeyia delagoensis* n. sp.   Cross-section of anterior part of the central
organs of proboscis.   × 55.

   au, auricle of pericardium. *c,* coelomic cavity. *epd,* epidermis. *gl,*
glomerulus. *lm,* longitudinal musculature. *m,* dorso-ventral muscle fibres.
nl, nerve-fibre layer. *rm,* ring musculature. *st,* stomochord. *va,* ventral
proboscis artery. *vp,* vermiform process. *vs,* ventral proboscis septum.

proboscis artery is not found in the septum; it runs from the top of
the stomochord freely through the coelom and directly to the epi-
dermis in a ventral direction (fig. 5).   According to Punnett this
vessel, called by him the ventral recurrent vessel of the proboscis,

runs in the upper edge of the septum in *W. bisulcata,* and according to his figure the ventral septum is exceedingly long in this species, reaching to nearly the top of the proboscis. The ventral coelomic blind-pouches are separated from each other throughout their whole length by this septum; they have the form of narrow finger-like canals, the epithelium of which is higher on the dorsal than on the ventral side (fig. 8). Their caudal end is at the same level as the caudal extremity of the ventral blind-sac of the stomochord; here near their posterior end the ventral pouches are quite embedded in the skeleton (fig. 6).

The right dorsal coelomic pouch ends blindly, but the left is continuous with a median end-sac (fig. 6) which opens by a left proboscis pore to the exterior (fig. 7). The proboscis pore has the form of an elongated fissure, the caudal end of which opens into the anterior neuropore. The end-sac does not extend beyond the caudal end of the pore and is surrounded by a thick limiting membrane, which probably contains blood-vessels (fig. 7).

It is impossible for the right and the left halves of the glomerulus to fuse in front of the pericardium or the stomochord because of the presence of the dorso-ventral septum. The glomerulus reaches as far in a frontal direction as the vermiform process, and that is not far in front of the stomochord. It covers the stomochord to the same extent as the pericardium and is found at the lateral side of these structures only; it even remains at some considerable distance from the dorsal and ventral mid-line. There is a triangular mass of cells extending for a considerable length along the dorsal side of the pericardium, but this mass does not seem to be a dorsal glomerulus as no blood-vessels could be detected in it; otherwise a dorsal glomerulus seems to be present in all Spengeliidae. On the coelomic side the glomerulus is covered by a rather thick layer of cells (fig. 5).

Immediately caudal to the mass of cells mentioned above, the free dorsal wall of the pericardium is thrown into irregular folds, which continue to where the pericardium attaches itself to the basal membrane of the epidermis, and only then the pericardium assumes its usual triangular form. Anteriorly the pericardium has two auricles as in other Spengeliidae. These auricles, however, are small; they do not reach to the top of the stomochord, but they are, as usual, surrounded by the anterior portion of the glomerulus (fig. 5). Anteriorly the pericardium is quite filled with a mass of cells; the middle portion, on the other hand, is quite empty except for a layer of cells along the wall; and in the posterior part, where the
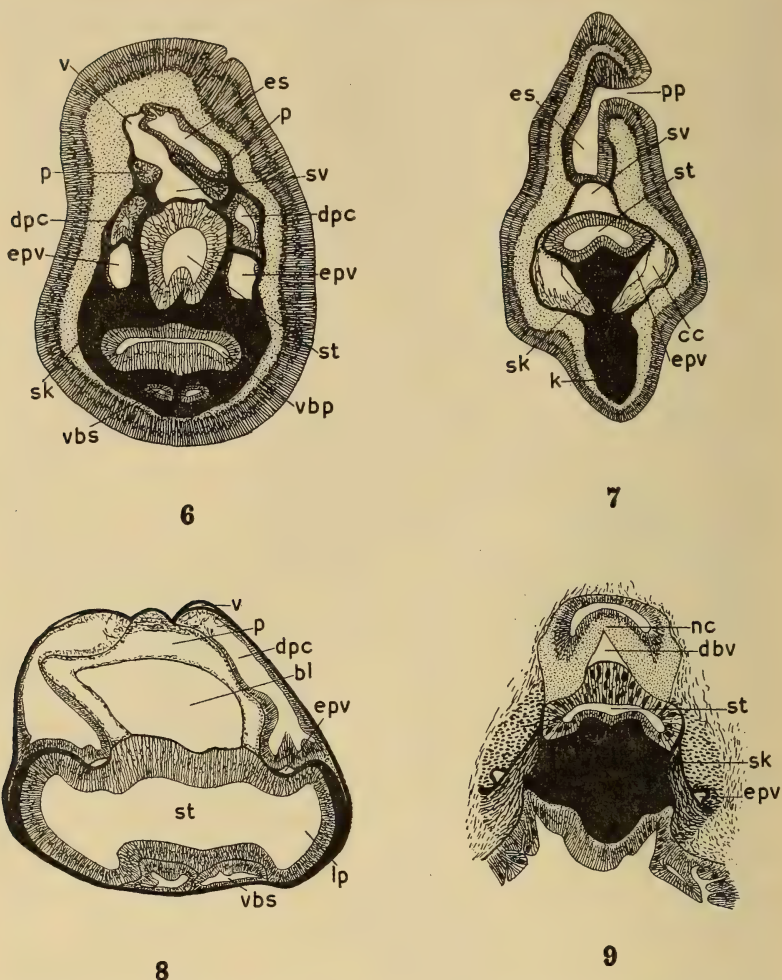
FIGS. 6–9.—*Willeyia delagoensis* n. sp.   6. Cross-section of proboscis neck. 7. Cross-section of proboscis neck caudal to that of fig. 6.   8. Cross-section of proboscis neck frontal to that of fig. 6 (epidermis being much damaged is not drawn in).   9. Dorso-median portion of a cross-section of anterior part of collar.   All × 45.

bl, central blood sinus.   *cc*, collar coelom.   *dbv*, dorsal blood-vessel.   *dpc*, dorso-lateral pouches of proboscis coelom.   *epv*, efferent proboscis vessel. *es*, end-sac.   *k*, keel of skeleton.   *lp*, lateral blind-pouch of stomochord. *nc*, nerve cord.   *p*, pericardium.   *pp*, proboscis pore.   *sk*, skeleton.   *st*, stomochord.   *sv*, sinus venosus.   *v*, proboscis vein.   *vbp*, ventral blind-pouch of stomochord.   *vbs*, ventral blind-sacs of proboscis coelom.

pericardium is connected with the epidermis, strong fibres are seen connecting the two side walls.

Near the posterior end of the pericardium the two proboscis veins unite at the right-hand side of the end-sac of the proboscis pore and then communicate with the wide sinus venosus (fig. 6). This communication pushes the posterior wall of the pericardium slightly forward so that in the sections the pericardium is visible at both sides of the blood-vessel.

As in all Spengeliidae, the stomochord is prolonged into a vermiform process (fig. 5), but in *W. delagoensis* this process is extremely short, being only 80 μ in extent. In *W. bisulcata* its length is about two-thirds of the length of the rest of the stomochord.

The anterior part of the stomochord is flattened; in cross-section it is oval in shape (fig. 5). Farther caudally it becomes more quadrangular. The lateral blind-pouches protrude somewhat in an anterior direction before they connect with the main middle part of the stomochord. The lumen, which is continuous throughout and begins near the top of the stomochord (fig. 5), becomes very wide and broad where the lateral blind-pouches connect with the middle part. The rather regular epithelial wall of the stomochord is very distinct in this region (fig. 8). More caudally, in the proboscis neck, the stomochord becomes first narrower but higher, then it is divided by the skeleton into a dorsal part, which is the neck of the stomochord, and a ventral blind-pouch, which is quite embedded in the skeleton (fig. 6). Except for the fact that ventrally an upgrowth of the skeleton causes an inpushing in its wall, this portion of the neck is nearly circular in cross-section (fig. 6), but farther caudally the neck becomes more depressed (figs. 7 and 9). In the neck part of the stomochord the epithelium contains numerous glandular cells, but in the body such cells could not be found.

Near the level of the middle of the lateral blind-pouches the limiting membrane at the sides of the stomochord increases in thickness, and this constitutes the most anterior end of the proboscis skeleton (fig. 8). The limiting membrane also becomes slightly thicker ventrally to the stomochord and the coelomic blind-sacs. More posteriorly these two lateral portions of the cup-shaped anterior end of the skeleton are united across the mid-line by the dorsal side of the cup, by which, as previously mentioned, the neck of the stomochord is separated from its ventral blind-pouch (fig. 6). Furthermore, the ventral wall of the cup has also thickened here considerably. At this point a dorso-median ridge of the skeleton cuts into the ventral wall

of the stomochordal neck.   In the dorsal part of the proboscis neck as well the limiting membranes have become very thick and the whole may be considered as one skeletal mass in which the efferent proboscis vessels, the dorso-lateral coelomic sacs, the posterior end of the pericardium, the sinus venosus, and the end-sac are embedded (fig. 6).   On the other hand, chondroid tissue, otherwise so well developed in the Spengeliidae, is hardly present in *W. delagoensis*. Caudally to the ventral blind-pouch of the stomochord, where the real skeleton becomes a solid structure, the more dorsally situated limiting membranes become thinner again.

Anteriorly, *i.e.* directly behind the anterior cup, the body of the skeleton cannot be distinguished from the keel, but near the anterior end of the collar coelomic cavities the skeleton is constricted by these cavities, so that body and keel may be easily distinguished here (fig. 7).   The keel has here a pair of wings dorso-laterally; it is large and broad though its dorso-ventral diameter is longer than its horizontal diameter.   At the level where the proboscis neck is attached to the collar, the connection between the body and the keel of the skeleton becomes broader again (fig. 16); here the crura also become visible in the middle of the skeleton, thus the fused crura are surrounded by secondary layers of skeletal material on all sides.   The skeleton now increases considerably in breadth, and the keel is much broader than high (fig. 9).   The crura reach to about half the length of the collar, and their extremities are found in the ventro-lateral angles of the buccal cavity, so that they surround this cavity for about two-thirds of its circumference.

Collar.—The epidermal zones of the collar are quite distinct and well differentiated from each other.   The epidermis of the anterior zone is rather low and it shows the usual nerve-fibre layer adjoining the basal membrane; this is followed by a layer of round nuclei of the nerve cells (fig. 10).   Externally to this is a dense and thick layer of elongated nuclei, which apparently belongs to the undifferentiated epidermis cells; this layer reaches nearly to half the thickness of the epidermis.   In the peripheral part of the epidermis are found some scattered, nearly round nuclei, which may belong to the glandular cells.   There are many glandular cells in the epidermis and they seem to extend over the whole thickness of this layer.   They are not swollen over their entire length, but the swollen part of the cells may be found at any level in the epidermis.   In some patches the swollen part of most if not all glandular cells is found near the surface, in others near the base of the epidermis.   In the latter case the oval

nuclei of the epidermis cells are displaced and found higher up in the cells.

The greater part of the epidermis of the collar consists of a very high epithelium formed by narrow, elongated cells (fig. 11). Here
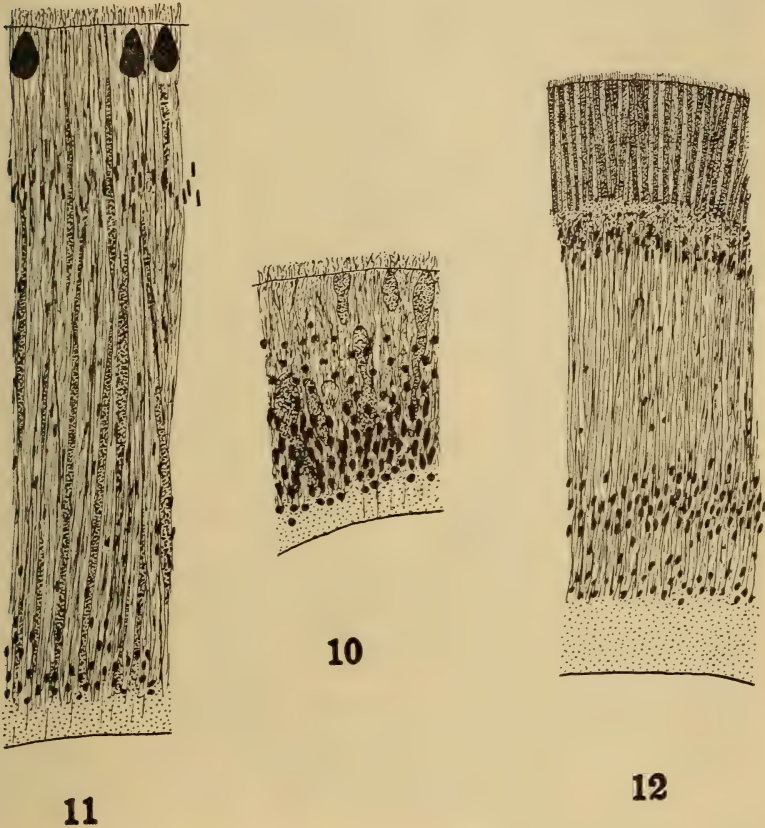
FIGS. 10–12.—*Willeyia delagoensis* n. sp.   10. Epidermis of the anterior zone of collar. 11. Epidermis of second zone of collar. 12. Epidermis of third zone of collar.   All × 220.

again are found the round nuclei of the nerve cells adjoining the nerve-fibre layer. A few very elongated nuclei are found scattered over the greater part of the epidermis, but these nuclei form a distinct layer at about one-quarter of the total thickness of the epidermis from the surface.   In this part of the epidermis there are many glandular cells, which are of two types. Some are as long as the epidermis is thick, their content is granular and stains with haematoxylin.   The

other type is found only near the surface, at least the part of the cell containing the glandular product; this product is homogeneous and stains with eosin. This zone is separated from the next one by a narrow deep groove in which the cells are similar to those just described except that they are not so elongated.

The next, rather narrow epidermal zone consists of a very characteristic epithelium (fig. 12). It is somewhat lower than that of the preceding zone, although the nerve-fibre layer is thicker. Next to the fibre layer are found the round nuclei of the nerve cells. The oval nuclei of the elongated epidermal cells are arranged in two distinct rows; one is found slightly external to the nerve-cell nuclei and the other higher up in the cells. Between these two rows of nuclei the cells are coloured uniformly blue by haematoxylin. External to the peripheral layer of nuclei and all starting at exactly the same level, all cells are filled with a fine granular substance that stains with eosin. The epidermis in this region therefore gives the impression that it is formed by two layers of cells. The cilia of this zone are very short, much shorter than in the two preceding zones. The zone is sharply delimited from the next one.

The posterior part of the collar, just in front of the circular nerve, is covered by a low epithelium containing many glandular cells and not differing from the epidermis of the trunk.

The external longitudinal musculature of the collar is well developed in the anterior half of the collar. It fills about half of the coelomic cavity between the epidermis and the gut (fig. 13). Its fibres, however, do not cross the body cavity in order to become attached to the wall of the gut at their caudal end. They remain in the same position near the epidermis throughout their whole length, and caudally they are fixed to the basal membrane of the epidermis. The external longitudinal musculature ends abruptly in about the middle of the collar (figs. 14 and 15).

According to Punnett there is no layer of circular muscles outside the longitudinal musculature. This is usually the case in Enteropneusta, as the external circular muscle of the collar is found inside the external longitudinal musculature. This external ring-muscle layer is very well developed in *Glandiceps*, but it is reduced in its extent in *Spengelia*. In *Willeyia* I find this layer very poorly developed and it is often interrupted (fig. 13). Furthermore, it is restricted to the anterior part of the collar.

The internal longitudinal musculature consists of coarser fibres than the external. It is, as in other Spengeliidae, fan-shaped.

Starting anteriorly at the dorsal side of the gut, where it is attached
to the folds of the limiting membrane in which the efferent proboscis
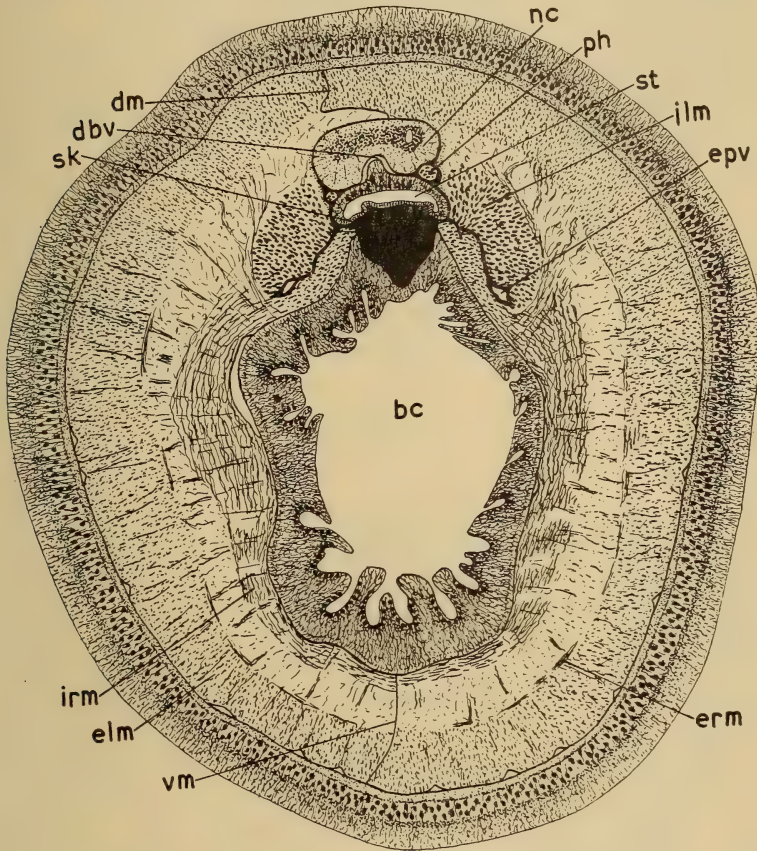vessels run (figs. 9 and 13), its fibres gradually spread out caudally

FIG. 13.—*Willeyia delagoensis* n. sp.   Cross-section of anterior part of collar.   × 35.

bc, buccal cavity.   *dbv*, dorsal blood-vessel.   *dm*, dorsal mesentery.
elm, external longitudinal musculature.   *epv*, efferent proboscis vessel.   *erm*,
external ring musculature.   *ilm*, internal longitudinal musculature.   *irm*,
internal ring musculature.   *nc*, nerve cord.   *ph*, top of perihaemal cavity.
sk, skeleton.   *st*, stomochord.   *vm*, ventral mesentery.

towards the ventral side, reaching the ventral mid-line about in the
middle of the collar near the posterior end of the external longitudinal
musculature (fig. 15).   In the posterior half of the collar the internal
longitudinal musculature is very strong, filling nearly the whole body
cavity.   It is divided up in irregular bundles by strands of radial

muscle fibres (fig. 15). In the anterior part of the collar there is a strong internal circular muscle layer round the buccal cavity (fig. 13).

The peripharyngeal cavities with their circular muscle fibres begin in the anterior part of the collar near the dorsal side of the buccal cavity, and as these cavities extend caudally they gradually surround a greater part of the buccal cavity and replace the internal ring musculature of the collar. The peripharyngeal cavities are rather irregular, because the limiting membranes at their outside and inside often coalesce locally, thereby interrupting the ring musculature in the cavities (fig. 14). On the other hand, the ventral end of the peripharyngeal cavities is not always closed, so that there are communications between these cavities, which in reality form part of the trunk coelom and the collar coelom. The ring muscles of the peripharyngeal cavities are then directly continuous with the internal ring musculature of the collar (fig. 15). In the anterior part of the collar the ventral boundary of the peripharyngeal cavities follows, as usual, the crura of the skeleton. From there the fold of the limiting membrane, in which the efferent proboscis vessel runs, extends somewhat more ventrally. The peripharyngeal cavities are closed off ventrally up to the end of the crura, and it is only beyond the crura that these communications occur.

At the dorsal side the peripharyngeal cavities do not extend below the perihaemal cavities; they do not even reach the latter cavities. Therefore, though there are ring-muscle fibres in the perihaemal cavities, there is nevertheless an interruption in the whole ring-muscle layer round the buccal cavity.

At their anterior end the perihaemal cavities diverge, so that here only the dorsal blood-vessel separates the collar nerve cord from the stomochord (fig. 13). The perihaemal cavities are also rather short, as they do not reach the anterior end of the collar (fig. 16). The septum between the right and the left perihaemal cavities is entire throughout their whole length.

The dorsal and ventral mesenteries stretch uninterruptedly throughout the whole length of the collar. In this species the ventral mesentery reaches the anterior end of the collar, whereas, according to Punnett, it is lacking in *W. bisulcata*.

At the front end of the collar nerve cord there is a deep anterior epidermal depression (the "vordere Vorhöhle" of Spengel). In *Spengelia alba* and *Glandiceps hacksi* there exists besides this anterior depression another inpouching of the epidermis just dorsal to the depression ("Epidermisgrube" of Spengel). Willey interpreted the
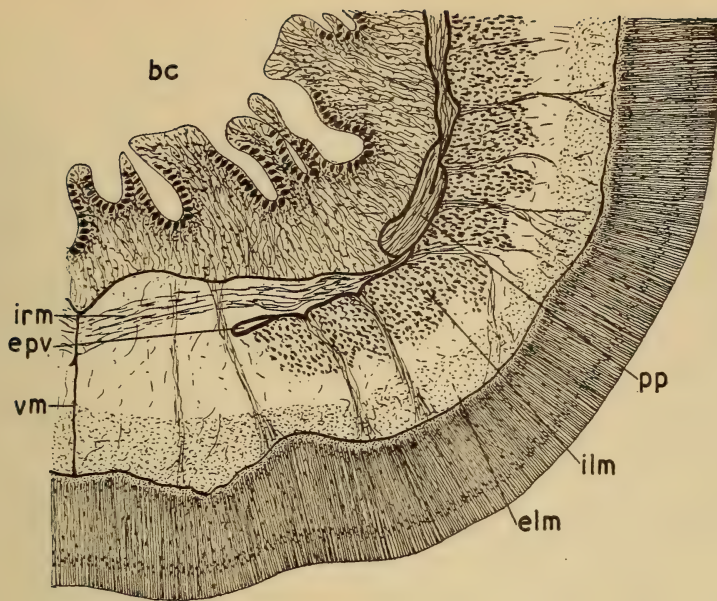
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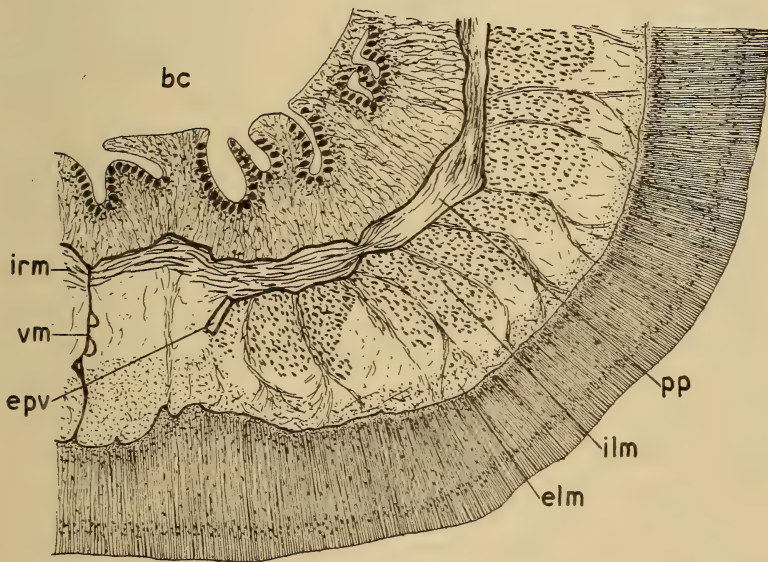
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Figs. 14, 15.—*Willeyia delagoensis* n. sp. 14. Ventro-lateral part of a cross-section of the collar. 15. The same, a few sections behind that shown in fig. 14. Both × 52.

bc, buccal cavity. *elm*, external longitudinal musculature. *epv*, efferent proboscis vessel. *ilm*, internal longitudinal musculature. *irm*, internal ring musculature. *pp*, peripharyngeal cavity. *vm*, ventral mesentery.

depression and the pouch as the same structure, which he called anterior neuropore. Spengel has pointed out that they are different structures, but he was of the opinion that the depression could not be called anterior neuropore, because its wall has an epidermal structure. However, it must be noted that the dorsal wall of the collar cord itself, if a central canal is present, may have an epidermal character, and as epidermis and collar cord are continuous, it is often very difficult to determine where the one stops and the other begins. Therefore I think it best to call the opening of the depression the neuropore; in a general way it corresponds with the neuropore of vertebrates. The depression itself is then the central canal that is present in the anterior part of the collar only. Thus, in addition to the neuropore, there may be an epidermal pouch dorsal to it, or the neuropore may be closed and the pouch present, or the pouch may be absent as in most Enteropneusta.

In his fig. 10 on plate xli Punnett draws the neuropore only, but in his fig. 15 on plate xlii the infolding of the epidermis is partially separated from the nerve cord and lying at its dorsal side. From this figure and fig. 16 one gets the impression that both pouch and neuropore are present in *W. bisulcata*, that the two are not completely separated from each other, and that the pouch is the deeper of the two.

However this may be, in *W. delagoensis* there is no pouch dorsal to the collar cord, but as a canal penetrates from the anterior end deep into the collar cord there is an open anterior neuropore (figs. 9, 13, and 16). A similar canal penetrating into the collar cord at its posterior end is hardly present; one may therefore conclude that the posterior neuropore is closed. The nerve cord shows only a few medullary cavities; in *W. bisulcata* it is quite solid. Punnett mentions a well-marked ridge projecting up towards the dorsal mesentery in the hinder portion of the nerve cord in *W. bisulcata*. No trace of such a ridge could be found in *W. delagoensis*. Punnett's statement that there are oesophageal nerves connecting the cord with the epithelium of the buccal cavity, called by him oesophagus, seems doubtful to me, as nothing resembling these nerves could be found either in *W. delagoensis* or any other enteropneust.

The collar canals fuse near their anterior ends with the first branchial sac, which here is already reduced to the size of a canal. Therefore what seems to be the collar canal is partly branchial pore, the inner wall of this canal being formed by the epithelium of the branchial sac (fig. 17). The dorsal or outer wall of the canal, which is slightly thinner than the side walls, shows a deep, inwardly directed fold.

The two lateral walls are very thick, and as in *Glandiceps talaboti* and a few other Enteropneusta the cells are so numerous and narrow that their nuclei, instead of being arranged in a single layer in the middle of the cells, form a dense mass occupying nearly the whole thickness of the epithelium (fig. 17).
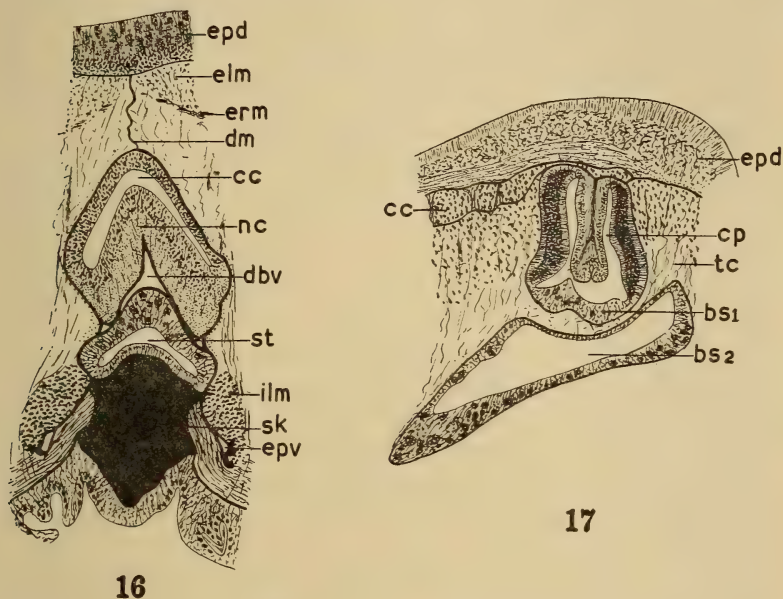
16   **17**

FIGS. 16, 17.—*Willeyia delagoensis* n. sp. 16. Dorso-median portion of a cross-section of collar slightly in front of that shown in fig. 9. 17. Cross-section of the collar canal. Both × 45.

> *bs*1, wall of first branchial sac. *bs*2, second branchial sac. *cc* (fig. 16), central canal of collar nerve cord. *cc* (fig. 17), collar coelom. *cp*, collar canal. *dbv*, dorsal blood-vessel. *dm*, dorsal mesentery. *elm*, external longitudinal musculature. *epd*, epidermis. *epv*, efferent proboscis vessel. *erm*, external ring musculature. *ilm*, internal longitudinal musculature. *nc*, collar nerve cord. *sk*, skeleton. *st*, stomochord. *tc*, trunk coelom.

The inner surface of the buccal epithelium is very irregular owing to the presence of numerous small folds penetrating into this epithelium (figs. 13, 14, and 15). There are numerous small glandular cells, which occur only in these folds and are absent from the free surface. They secrete a slimy substance in the form of long threads. The threads of the separate cells coalesce into thicker strands, which emerge from the folds into the buccal cavity. The whole is reminiscent of a byssus gland of a Lamellibranchiate.

Trunk.—The first few branchial sacs are situated far in front of

their external apertures, so that these sacs are drawn out into long canals leading to the pores (fig. 17). The total number of gills is 144 on each side of the body; Punnett, unfortunately, does not give the number of gills in *W. bisulcata*. The latter species is characterised by the very short branchial bars, so that the branchial part of the pharynx is much smaller in the cross-section than the digestive part. Although the tongues are also short in *W. delagoensis*, the two parts of the pharynx are of about equal size, the branchial part being even a little larger than the digestive part. There is no indication even of parabranchial ridges separating the two parts of the pharynx.

According to Punnett the first three gill pouches of *W. bisulcata* are dorsally confluent, forming a chamber with a single external pore into which opens the collar pore. A similar fusion of a few of the anterior gill pouches is known in some other Enteropneusta. In *W. delagoensis*, however, the anterior gill pouches have separate pores, though the first and second pores touch each other. These pores open into a very deep and narrow sublateral groove. In the middle portion of the branchial region the groove becomes shallower (fig. 18), but in the posterior part it is very deep again (fig. 19). This accounts for one's inability to see the small branchial pores when studying the external features of the animal.

The absence of synapticula has been already mentioned as a character of the genus *Willeyia*.

The tongues are short and very thick, so that they fill up nearly the whole of the branchial sacs (fig. 18). This is due to the fact that the ciliary epithelium covering the anterior and posterior surfaces of the tongues and septa is very high. It has the same character as the epithelium of the collar canals, consisting of very high and narrow cells, the nuclei of which fill up nearly the whole thickness of the epithelium instead of forming a single row in the middle. In *Glandiceps talaboti* the collar canals have a similar epithelium, but the gills have the low ciliated epithelium as in most Enteropneusta. On the other hand, *Harrimania kupfferi* and a few other species belonging to the Harrimaniidae have high epithelium like *W. delagoensis* both in the collar canals and the gills. The nature of this epithelium in *W. bisulcata* is not mentioned by Punnett. The back of the septa, facing the pharyngeal lumen, is covered by a thin epithelium, while that on the back of the tongues is higher.

Following the last gill there are two more rudimentary gills, each consisting of a small sac and tongue only but having no opening to the exterior (fig. 19).

The digestive part of the pharynx is covered by a very high epithelium, the inner surface of which shows many irregular folds (fig. 18). In the anterior part of the oesophagus the epithelium is much lower and without folds (fig. 19).

The longitudinal musculature of the branchial region as well as that of the genital region, as far as this could be studied in this specimen, is very well developed. It fills nearly the whole of the body cavity, leaving only a narrow space around the intestine. There is no special thickening of this musculature near the ventral mid-line; it was only found that the fibres may be more crowded there (fig. 18). An internal ring muscle around the ventral pharynx is well developed. The ventral nerve cord projects very far inwards, a fact which has also been observed by Punnett.

According to Punnett the branchial region of *W. bisulcata* is probably devoid of gonads, or if gonads occur here then they are found only in the most posterior portion. In this respect *W. delagoensis* shows a marked difference from *W. bisulcata*. In the former species the first gonopore is found near the 34th branchial pore, which means that the row of gonads starts at about a quarter of the total length of the branchial region away from the collar. Punnett's specimen of *W. bisulcata* was a male; the only specimen of *W. delagoensis* available is a female, but as the extent of the gonads is not known to show sexual dimorphism in any other enteropneust it seems most unlikely that such a difference should occur here.

The first gonads are small and have a well-pronounced gonoduct, opening in the sublateral groove at the ventral side of the branchial pores (fig. 18). Caudally they increase in size, thus gradually enveloping the intestine as they extend towards the ventral side (fig. 19). The specimen was broken off in the anterior part of the genital region, and, as yet, the gonads had not increased sufficiently in size to cause the body wall to protrude in the form of genital folds. As in *W. bisulcata*, there are no median gonads, although the gonads project forward around the posterior gill slit so as to appear on its medial side (fig. 19).

Diagnosis of Willeyia delagoensis *n. sp.*

In the fixed state the proboscis is 15 mm. long and its greatest width is 5 mm., the collar is 5 mm. long and broader behind than in front, the branchial region is about 3 cm. long (the only specimen collected is broken off near the frontal end of the genital region).
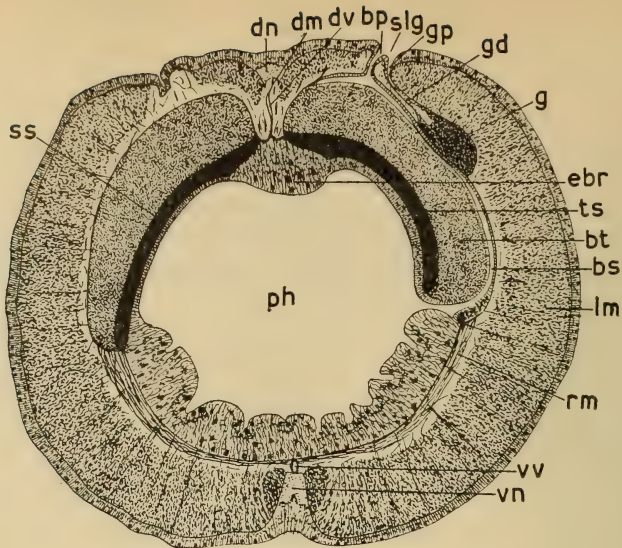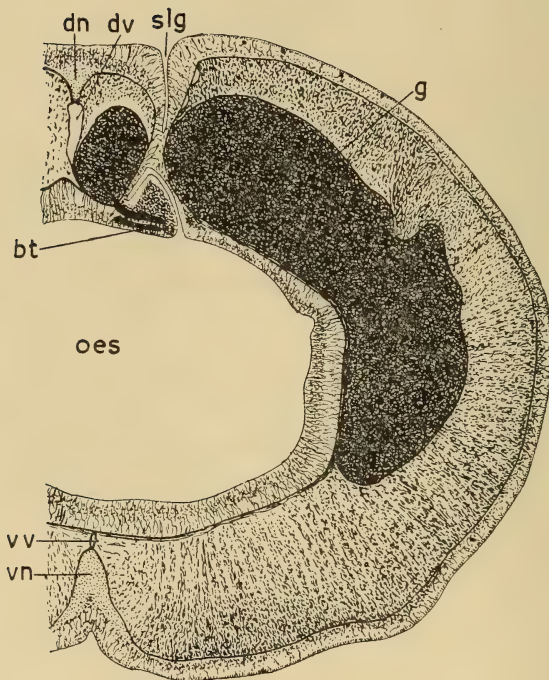
FIGS. 18, 19.—*Willeyia delagoensis* n. sp.   18. Cross-section of the branchial region, composed of several sections, in order to show a tongue at one side and a septum at the other; in reality two tongues are opposite one another. The section is not through the middle of the tongue, showing the tongue coelom, but through the anterior or posterior end of the tongue and the septum in order to show the thick layer of crowded nuclei.   ×20.   19. Cross-section of the posterior end of the branchial region.   ×34.

bp, branchial pore. *bs*, branchial sac. *bt*, tongue. *dm*, dorsal mesentery. *dn*, dorsal nerve cord. *dv*, dorsal blood-vessel. *ebr*, epibranchial ridge. *g*, gonad. *gd*, gonoduct. *gp*, genital pore. *lm*, longitudinal musculature. *oes*, oesophagus. *ph*, pharynx. *rm*, ring musculature. *slg*, sublateral groove. *ss*, septal skeletal bar. *ts*, tongue skeletal bar. *vn*, ventral nerve cord. *vv*, ventral blood-vessel.

The proboscis is flattened, with a distinct groove in dorsal and ventral mid-lines. The collar and the branchial region are cylindrical, no trace of genital wings. The living animal is of a pure white colour.

Proboscis.—No special glandular cells in the epidermis of the proboscis. Circular muscle layer of the proboscis about as thick as the nerve-fibre layer; longitudinal muscle fibres do not show any special arrangement. The proboscis coelomic cavity extends nearly to the top of the proboscis and is separated into right and left parts of unequal size by a dorso-ventral septum formed of connective tissue and muscle fibres. Ventral proboscis septum does not extend farther forward than the top of the stomochord. Ventral proboscis artery free from ventral septum. Ventral coelomic blind-sacs separated from each other throughout their whole length. Left dorsal coelomic sac communicates with a median end-sac; proboscis pore on the left side. Right and left half of the glomerulus not connected in front of the pericardium or stomochord nor at their dorsal or ventral sides; frontal end of glomerulus at both sides surrounding the auricles of the pericardium. No dorsal glomerulus. Pericardium with two short auricles. Vermiform process of stomochord very short. Stomo-chord with wide lumen, extending to near the top, very wide where the lateral pouches connect with central part. Hardly any chondroid tissue, but skeleton very well developed, with deep anterior cup; just behind the cup the body of the skeleton is nearly separated from the keel by the collar coelomic cavities, farther caudally the skeleton becomes broader, and keel and body are indistinguishable. Crura reach to about half the length of the collar and surround the buccal cavity for about two-thirds of its circumference.

Collar.—First zone of collar epidermis low, second zone with very thick epithelium and small superficial glandular cells, third zone with thick epithelium and a distinct superficial layer of glands, fourth zone low like trunk epithelium. External longitudinal musculature in anterior half of collar only, attached to the epidermis at both ends. Thin external circular muscle layer, internal circular muscle layer well developed ventrally to the peripharyngeal cavities; these cavities do not reach the perihaemal cavities, the latter with a layer of circular muscle fibres. The perihaemal cavities quite sepa-rated from each other, their anterior ends diverge and do not reach the anterior end of the collar. Dorsal and ventral mesenteries complete. Central canal in anterior end of collar cord only; in the rest a few medullary cavities. No epidermal pouch dorsal to the anterior neuropore. No dorsal ridge on the nerve cord. Collar

canals with thick epithelium that is almost entirely filled by the crowded nuclei.

Trunk.—Number of gills 144. Branchial part of the pharynx about equal in size to digestive part. No parabranchial ridges. The first gills open to the exterior by separate pores, though the first and second pores touch each other. Sublateral groove narrow and deep in anterior and posterior part of branchial region, shallower in middle portion. No synapticula. Ciliated epithelium of tongues and septa very high, with thick and dense layer of nuclei. Epithelium of digestive part of the pharynx very high, much higher than that of anterior portion of oesophagus. Row of gonads begins at one-quarter the length of the branchial region. No median gonads.

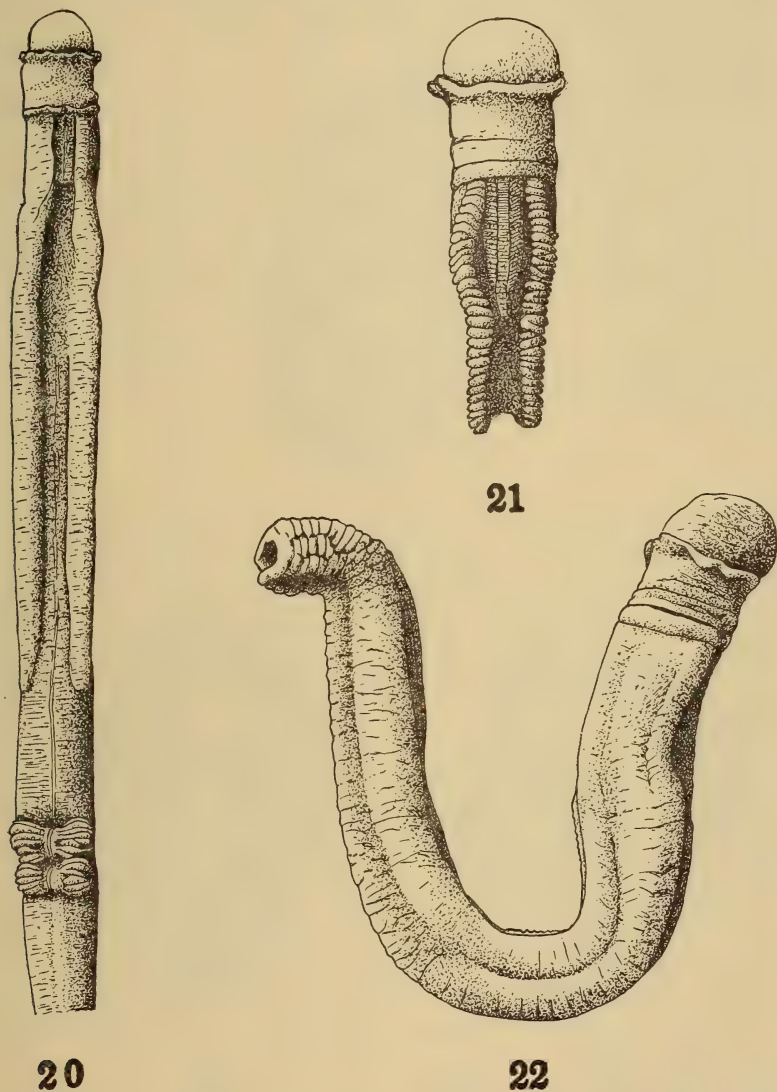## GLOSSOBALANUS ALATUS n. sp.

External Features.

In the material collected at Inyack in July 1934 was found a single specimen of a *Glossobalanus*, broken off near the hind end of the genital region (fig. 22). Two other specimens were found in November 1935, one of which was broken off shortly behind the liver region (fig. 20) and the other in the genital region (fig. 21). The specimens 1 and 3 were cut into serial sections.

On account of its rather broad genital pleura it might be thought to be a species of *Balanoglossus*, but its internal anatomy clearly shows it to be a *Glossobalanus*. *Gl. marginatus* seems to have even broader genital wings as can be seen from Meek's figures, but otherwise the genital pleura are not very pronounced and set off from the body in the species of *Glossobalanus*. In *Gl. mortenseni* and *Gl. ruficollis* the pleura are absent altogether.

These genital wings are well set off from the body itself (fig. 22); they start shortly behind the collar and reach their maximum width near the end of the branchial region (figs. 36 and 37). In the genital region they show hardly any decrease in size and they end well in front of the liver region, so that, as in some species of *Balanoglossus*, a transitional region is formed between the genital and hepatic regions. In most species of *Glossobalanus* the branchial region is short compared with that of *Balanoglossus*. In *Gl. alatus* it is extremely short, measuring only 3·3 mm. of a total length of about 28 mm. for the whole branchio-genital region, or 34·5 mm. for the distance between the collar and the liver region (fig. 20). This is relatively and also absolutely shorter than in any other species of

Glossobalanus, with the exception of *Gl. ruficollis*, in which much larger animal the branchial region measures about 8 mm. in a full-

FIGS. 20–22.—*Glossobalanus alatus* n. sp. 20. Dorsal view, × 3. 21. Dorsal view, × 5. 22. Lateral view, × 6.

grown specimen; the specimens of *Glossobalanus alatus* are full grown also, as is shown by the well-developed gonads. Moreover,

Spengel has shown that during growth the branchial region becomes relatively shorter in comparison with the genital region, but even in the largest specimens of *Gl. minutus* Spengel found the branchial region to have a length of one-fifth of the whole branchio-genital region; here in *Gl. alatus* it is only one-tenth.

The epithelial ridges of the trunk are not so pronounced as in other species, e.g. *Gl. marginatus*. The proboscis is 2 mm. long and 3 mm. broad; the collar is 3 mm. long by a greatest width of 3·8 mm. The total length of the genital pleura is 28 mm. and of the transitional region 6·5 mm. The liver region is very short, 3·7 mm. only, and well delimited both at the anterior and posterior ends. Only 14 liver saccules could be seen from the outside, and they are all subequal in size. Over the whole length of the trunk, as far as could be seen from the specimens, a regular row of small depressions is clearly visible at both sides of the ventral nerve cord.

Internal Anatomy.

Proboscis.—The epidermis of the proboscis is of uniform thickness, no longitudinal striae, as shown in *Gl. mortenseni*, being visible either on the surface or in cross-sections. The nuclei are scattered in the basal half of the epidermis outside the nerve-fibre layer; a few nuclei may be found in the peripheral part (fig. 23). Many small glandular cells occur in the peripheral part of the epidermis between the ordinary epithelial cells.

A thin circular muscle layer, about half as thick as the nerve-fibre layer, is found underneath the basal membrane of the epidermis. Though no membrane could be detected along the inner side of the circular musculature as in *Gl. crozieri*, there are in this place many cells which, however, do not form a well-defined epithelium. In *Gl. mortenseni* both the membrane and the cells between the circular and the longitudinal musculature are absent. The longitudinal muscle fibres are accumulated in the central part round the proboscis cavity; in the peripheral part a delicate network of connective tissue is found with only a few isolated muscle fibres. The longitudinal muscle fibres show a tendency to form bundles, and these bundles again are more or less radially arranged, especially in the caudal part of the proboscis near the central organs (fig. 23). A dorso-ventral muscle plate, found in other species, is absent in *Gl. alatus*.
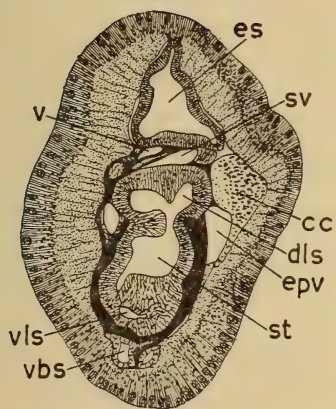
The central proboscis cavity is small and is confined only to the posterior part of the proboscis round the central organs. It is also
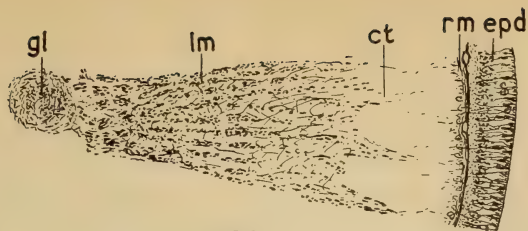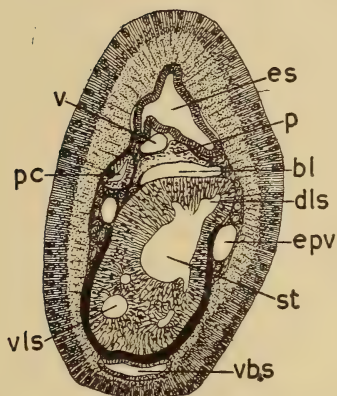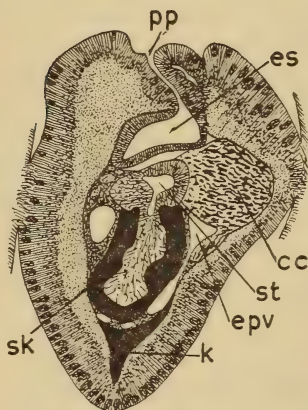
FIGS. 23–26.—*Glossobalanus alatus* n. sp. 23. Dorso-median segment of a cross-section of proboscis. 24. Cross-section of proboscis neck. 25. The same, a few sections caudal to that of fig. 24. 26. Cross-section of proboscis neck where it fuses with the collar. All × 52.

bl, central blood space. *cc*, collar coelom. *ct*, connective tissue. *dls*, dorso-lateral blind-pouch of stomochord. *epd*, epidermis. *epv*, efferent proboscis vessel. *es*, end-sac. *gl*, anterior extremity of glomerulus. *k*, keel of skeleton. *lm*, longitudinal musculature. *p*, posterior end of pericardium. *pc*, posterior end of right dorso-lateral proboscis coelom. *pp*, proboscis pore. *rm*, ring musculature. *sk*, skeleton. *st* (figs. 24, 25), main lumen of stomochord. *st* (fig. 26), neck of stomochord. *sv*, sinus venosus. *v*, proboscis vein. *vbs*, ventral proboscis blind-sac. *vls*, ventro-lateral blind-pouch of stomochord.

very narrow, at the dorsal side it is even quite suppressed, the glomerulus and the pericardium being here in direct contact with the muscular and connective tissues surrounding the cavity (figs. 23 and 27). The central cavity is therefore crescent-shaped in cross-section.

The ventral proboscis septum is very short. It connects with the basal membrane of the epidermis only at the level of the lateral blind-pouches of the stomochord; its attachment to the stomochord extends slightly more frontally. The ventral proboscis artery runs along its anterior edge (figs. 28, 29, and 30). The ventral coelomic blind-sacs of specimen 1 are very small, being found in only 7 sections each $10\,\mu$ thick. When they have disappeared in the cross-sections one finds in the same position between the stomochord and the epidermis a group of cells without a lumen. These cells are continuous with the wall of the blind-pouches. A few sections more caudally, at the anterior end of the skeleton, there appears a median lumen in this group of cells that becomes rather wide and can be considered as a reappearance of the ventral blind-sacs (fig. 24). A few sections farther on, this lumen is divided into two parts by the keel of the skeleton (fig. 25), and when the keel becomes larger this continuation of the ventral blind-sacs ends (fig. 26). In specimen 3, however, an interruption of the ventral coelomic blind-sacs does not occur; after fusing with each other, they continue backwards between the body and the keel of the skeleton and disappear at the level where the keel increases in size.

The right dorsal coelomic sac ends blindly as usual, only the left being in communication with the rather irregular, triangular, median end-sac. The proboscis pore is also median (figs. 24, 25, and 26). After the two proboscis veins have fused with each other, they run along the right-hand side of the end-sac and unite with the sinus venosus along the caudal end of the pericardium (figs. 24 and 25). The sinus venosus exhibits no particular features.

The anterior end of the stomochord is, except for some protrusions into the central blood space, nearly cylindrical at first (fig. 27); then the dorsal side becomes flatter, so that the stomochord is here broader than high (figs. 28, 29, and 30). The lumen begins as a small cavity near the top and is continuous throughout; in specimen 3 it is a straight canal, but in specimen 1 it is often twisted, so that it may be cut three times in one section. Two ventro-lateral blind-pouches of the lumen occur in the ventral dilation of the stomochord (fig. 24). In specimen 1 each of these blind-pouches has a separate narrow connection with the main lumen that has here increased considerably

in size; they are also connected with each other by an equally narrow canal (fig. 25). As in specimen 3, these narrow canals are interrupted, the pouches are here not connected with each other nor with the main lumen. Besides the ventro-lateral blind-pouches, the stomochord of specimen 1 has also dorso-lateral blind-pouches, which otherwise are found only in some species of *Balanoglossus* (fig. 24). They have a common communication with the central lumen (fig. 25). Caudal to the level of fig. 25 the lumen in the dorsal part of the stomochord disappears, leaving only the main central lumen in the body. The tissue of this dorsal part remains, however, and is continuous with the neck of the stomochord. A new lumen appears here which is connected with the main lumen of the body by a narrow canal (fig. 26). The dorso-lateral blind-sacs are altogether absent in specimen 3. There are hardly any glandular cells in the stomochord except near its opening into the buccal cavity.

The pericardium reaches as far anteriorly as the stomochord in specimen 1; in specimen 3 the stomochord extends in front of the pericardium. In the anterior part of the pericardium there is a thick layer of muscle fibres along its ventral wall (fig. 27), but even before the pericardium has become attached to the epidermis these fibres have disappeared (fig. 29). The pericardium of specimen 1 shows a remarkable peculiarity that has never before been recorded in any other enteropneust. Always in Enteropneusta the pericardium is a completely closed vesicle, and no conclusion can be drawn as yet as to whether it originates from the coelom or not. It is therefore of interest that the pericardium of this specimen communicates with the proboscis coelom. In its anterior part the pericardium is closed off completely from the coelom, and the limiting membrane between the two is uninterrupted (fig. 27). But where the pericardium wall begins to extend towards the dorsal side in order to attach itself to the basal membrane of the epidermis, there appears a large opening in the dorsal wall (fig. 28), which opening extends along the right wall of the pericardium when the latter is in contact with the epidermis (fig. 29). Further caudally the pericardium again is completely closed off from the proboscis coelom (fig. 30). Through this opening the connective tissue and even the longitudinal muscle fibres of the proboscis coelom enter the pericardium. Such an opening has never been described in any other enteropneust before; it may be quite accidental in this specimen, but it is certainly not an artefact.

The glomerulus covers and surrounds the anterior ends of both the pericardium and the stomochord. The glomerulus soon becomes

smaller and then disappears at the dorso-median side (fig. 27). At
this point it reaches nearly to the mid-ventral line, but as the stomo-
chord is small and the pericardium extends far ventrally, the greater
part of the glomerulus covers the sides of the pericardium (figs. 27
and 28).

The anterior end of the skeleton is U-shaped in cross-section and
surrounds the ventral dilation of the stomochord (fig. 24). It becomes
thicker when this dilation gets smaller (figs. 25 and 26), until it becomes
more of a solid mass (fig. 31). But also in this region it seems to be
composed of two symmetrical halves due to the presence of a dorso-
median groove and a deep narrow fissure extending from this groove
deep into the body of the skeleton. In specimen 1 it is only in a
few sections, near the posterior end of the keel, that the body appears
as a real unit. The keel begins shortly behind the anterior end of
the skeleton (fig. 25); it is small at first but then suddenly increases
in size and it extends over nearly the whole length of the body;
caudally it ends rather abruptly. A very characteristic feature is
the separation of the keel from the primary body of the skeleton
nearly throughout its whole length. Only with the secondary body,
formed by the fusion of the crura, is the keel firmly united. Also,
extensions from the collar coelom and side branches from the efferent
proboscis vessels penetrate in between the body and the keel (fig. 26).
Coelomic tissue also invades the sides of the body itself, thus giving
it the appearance of chondroid tissue (fig. 31), which otherwise is only
poorly developed. In specimen 1 the secondary body, formed by
the union of the crura, begins immediately behind the posterior end
of the keel, but in the older specimen 3 the secondary body is much
longer and the keel extends along it over a considerable distance.
The free crura are short and thick, and extend straight in ventro-
lateral direction from the body. They surround the buccal cavity for
about half its circumference.

Collar.—The five epidermal zones of the collar are well demarcated
(fig. 32). The first, rather broad, zone is well supplied with large
vacuoles. The nuclei are found just outside the nerve-fibre layer,
below the vacuoles, but as many vacuoles reach as far down as the
nerve-fibre layer, the nuclear layer is rather irregular. A few scattered
nuclei may be seen in the more peripheral part of the epidermis.
There are small, short glandular cells filled with a granular substance
near the surface, and some narrow elongated glandular cells extending
deeper down in the epidermis, the content of which is darkly stained
and homogeneous.
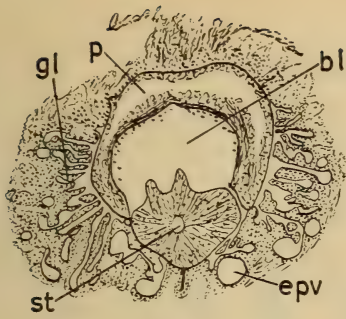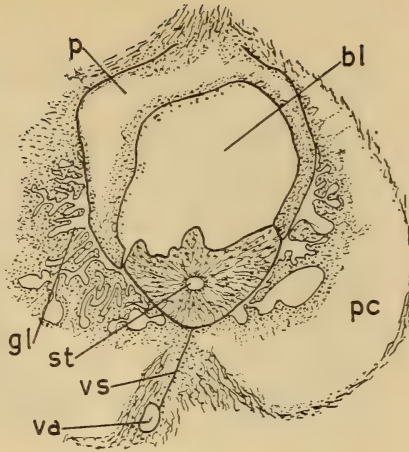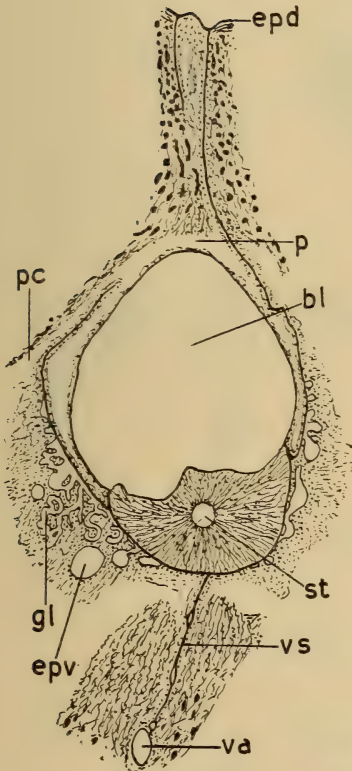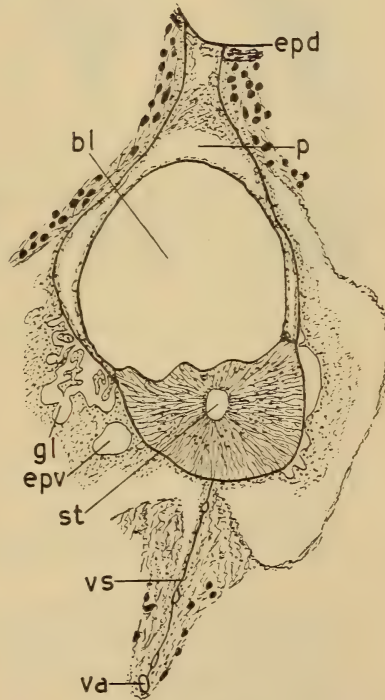
FIGS. 27–30.—*Glossobalanus alatus* n. sp. 27. Cross-section through anterior part of proboscis organs. 28. The same, caudal to that of fig. 27. 29. The same, caudal to that of fig. 28. 30. The same, caudal to that of fig. 29. All × 100.

bl, central blood space. *epd*, basal membrane of epidermis. *epv*, efferent proboscis vessel. *gl*, glomerulus. *p*, pericardium. *pc*, proboscis coelom. *st*, stomochord. *va*, ventral proboscis artery. *vs*, ventral proboscis septum.

The second zone is narrower than the first, but the epithelium is about twice the thickness of that of the first zone. It consists of very elongated, non-vacuolated cells, the oval nuclei of which are situated in a row about in the middle of the cells. A layer of
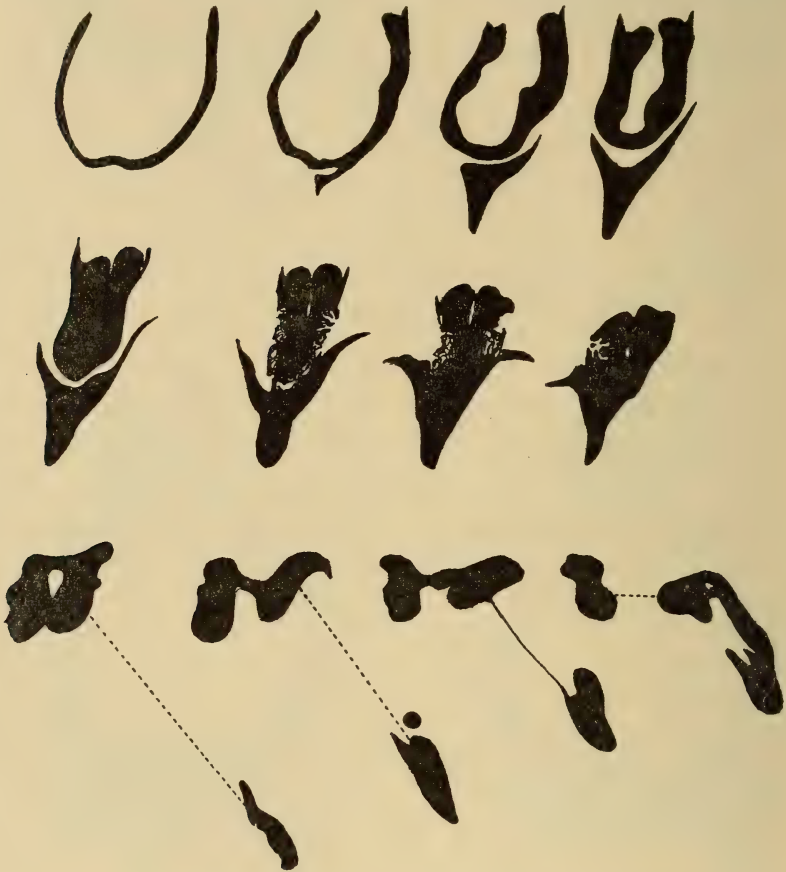
FIG. 31.—*Glossobalanus alatus* n. sp. Every fifth cross-section, each 10 μ thick, of the proboscis skeleton. × 55.

round nuclei, apparently belonging to the nervous cells, lies just outside the nerve-fibre layer. Very small glandular cells with a granular content are found just beneath the outer surface of the epithelium.

The third zone is a little broader and as thick as the second, and has a few vacuoles in the peripheral part of the epithelium. The whole basal half is filled with nuclei, and judging by the number of

nuclei the cells must be very numerous and consequently narrow. This is the main difference between the second and the third zones. There are a few small glandular cells near the free surface.

The fourth zone, forming the circular groove, is consequently narrow and consists of a low epithelium. It is a striking fact that the nerve-fibre layer in this zone is very thin. Just above this layer is a single row of nuclei, and a few more nuclei are scattered throughout the epithelium. The cells must be rather broad here. There are large vacuoles and long thin glandular cells that reach to near the basal membrane. The cilia of this zone are much shorter than those of the other zones.

The fifth and last zone is very similar to the first. The epithelium, which is very vacuolated throughout its whole thickness, contains small glandular cells near the surface. The majority of the nuclei are found in the basal part underneath the vacuoles, only a few scattered nuclei being observed nearer the surface.

As in other species of Enteropneusta, the dorsal mesentery of the collar begins at the first or the second dorsal nerve root and is complete from there to the posterior end of the collar. No trace of this mesentery could be found in front of the first nerve root. A ventral mesentery, on the other hand, is almost entirely missing, it being only present over a short distance in the posterior end of the collar. Furthermore, there are a few connections between the ventro-median blood-vessels running along the intestinal wall and along the inner side of the epidermis.

The collar canals have the usual form with only the dorsal wall folded inwards.

As in *Gl. mortenseni* the medullary cord has a continuous central canal, but *Gl. alatus* differs from that species in the absence of an anterior epidermal pouch dorsal to the anterior neuropore. There are two dorsal nerve roots in specimen 1 and four in specimen 3, and although the central canal sends a branch into these roots, they are solid for the greater part of their length (fig. 33). The supraneural blood-vessels are quite conspicuous.

The perihaemal cavities begin at the level of the proboscis pores. Their anterior ends are adjacent to each other except where they are separated by the dorsal blood-vessel. The mesentery separating the two perihaemal cavities from each other is complete over the whole length of the collar.

The epithelium of the buccal cavity shows a nearly uninterrupted layer of glandular cells near the free surface. Their contents are

darkly stained by haematoxylin. The nuclei are found just under-
neath the glands (fig. 33).

Trunk.—A mid-ventral groove accompanying the ventral nerve
cord is hardly indicated and then only in the anterior part of the
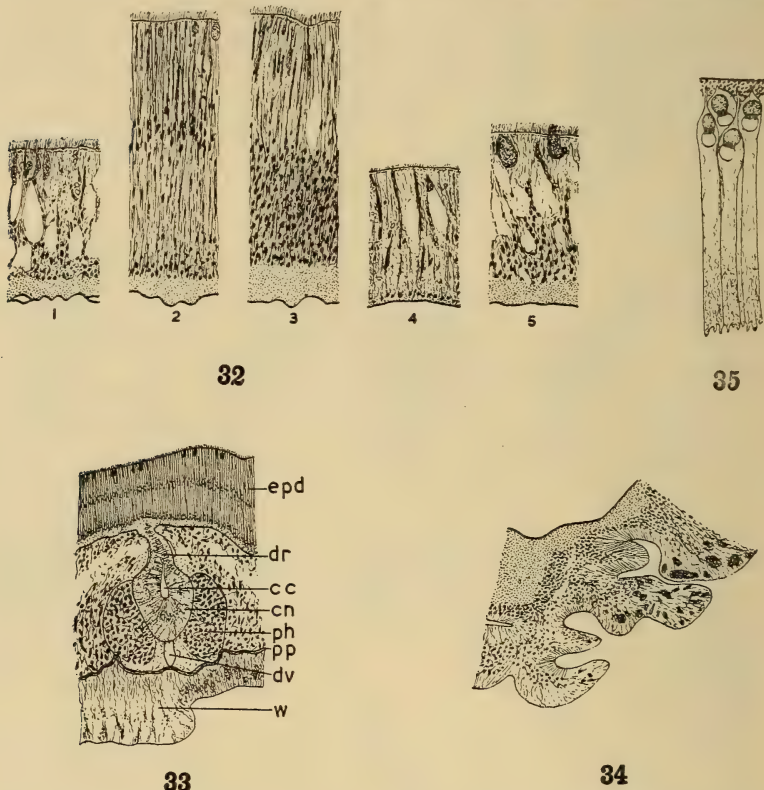
FIGS. 32–35.—*Glossobalanus alatus* n. sp.   32. Sections of the five epidermal zones
of the collar. × 180.   33. Cross-section of the dorso-median part of the
collar. × 40.   34. A more or less tangential section through the epidermis
of the branchio-genital region near the mid-ventral line, showing the ventral
nerve cord and two epidermal pits. × 77.   35. A few of the cells forming
the bottom of the epidermal pits. × 735.

cc, central canal. *cn*, collar nerve cord. *dr*, dorsal nerve root. *dv*, dorsal
blood-vessel. *epd*, second epidermal zone. *ph*, perihaemal cavity. *pp*,
peripharyngeal cavity. *w*, wall of buccal cavity.

branchial region. The dorso-median groove is also shallow and
disappears altogether at the beginning of the genital wings. On the
other hand, the branchial groove is deep over the whole length of the
branchial region (figs. 36 and 37); anteriorly it is very narrow, but it
widens out at the beginning of the genital wings.

The epidermis of the trunk contains a great many glandular cells of two types. The larger ones are granular or at least not homogeneous and stained with haematoxylin; the smaller ones contain a homogeneous substance stained with eosin. Both types of glandular cells are found over the whole thickness of the epidermis, although they are more numerous near the surface (fig. 36).

There is a row of peculiar epidermal depressions with a conspicuous epithelium at both sides of the ventral nerve cord extending throughout the whole length of what there was of the trunk in the two specimens available. I have never seen anything similar in other Enteropneusta, nor has it been described by other authors. Judging from Spengel's figures these depressions might be present in *Gl. hedleyi*, but in his very accurate and detailed description of that species Hill does not mention nor figure them. In *Gl. marginatus* Meek mentions a paired series of pigment spots which lie on each side of the ventral median line of the branchio-genital region. In their position these pigment spots quite agree with the epidermal pits of *Gl. alatus*; in the latter species, however, no pigment was observed.

These pits are more or less oval depressions each of which is separated from the next one by a ridge of much higher ordinary epidermal epithelium (figs. 36–41). As this epithelium overhangs the depressions on all sides, they appear like little niches, opening to the surface by a rather narrow slit (fig. 34). In that part of the animal that was bent (fig. 22), more or less tangential sections of the epidermis near the ventral mid-line were obtained; here it could be seen that the middle part of the bottom of the depression is slightly raised (fig. 34). The product of the glandular cells of the epidermis fills the depressions to some extent; at any rate, the epithelium here is to a large extent covered by a substance that seems to be derived from these glandular cells, viz. from those that stain with haematoxylin. Histologically the epithelium forming the bottom of these pits differs greatly from the rest of the epidermis. There are no glandular cells, but only regular columnar cells, slightly swollen near the base where the nuclei are found (fig. 35). The free surface of the cells shows small protrusions, on some of which a small black point could be seen. The protoplasm is accumulated in the peripheral part of the cells, while in the basal half the protoplasm is only located along the cell walls. The nuclei are very characteristic. In the nuclei of the ordinary epidermis cells and also in those of the nerve cells the usual chromatin network is present, but in these cells the nuclei have the form of an 8, the middle of which is very darkly stained, so most of the chromatin
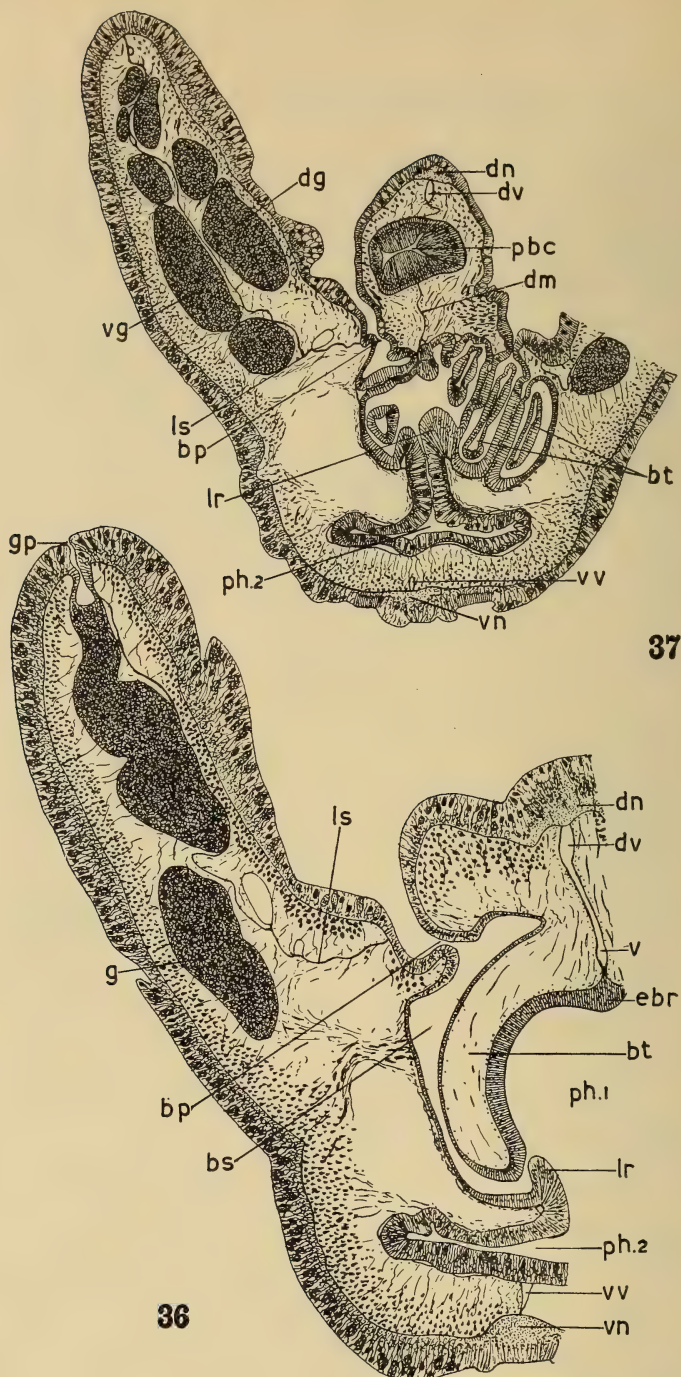
FIGS. 36, 37.—*Glossobalanus alatus* n. sp.   36. Cross-section of the branchial region. ×52.   37. Cross-section near the posterior end of the branchial region. ×34.

bp, branchial pore. *bs*, branchial sac. *bt*, branchial tongue. *dg*, dorsal gonad. *dm*, dorsal mesentery. *dn*, dorsal nerve cord. *dv*, dorsal blood-vessel. *ebr*, epibranchial ridge. *g*, gonad. *gp*, gonopore. *lr*, limiting ridge. *ls*, lateral septum. *pbc*, anterior blind-sac of the postbranchial canal. *ph1*, branchial part of pharynx. *ph2*, digestive part of pharynx. *v*, blood-vessel from branchial tongue. *vg*, ventral gonad. *vn*, ventral nerve cord. *vv*, ventral blood-vessel.

seems to be accumulated here. Below this dark part and at the side towards the basal membrane the nucleus is slightly and evenly stained; there may be chromatin here, but it does not form a network. The peripheral part of the nucleus looks like a vacuole with a thick wall, which is much thicker than a normal nuclear membrane. In this part the nucleus is not stained at all. The base of each cell is continued into a thread-like process that penetrates into the nerve-fibre layer, but nowhere could it be observed that this thread goes right through the nerve-fibre layer in order to attach itself to the basal membrane, a condition easily seen in ordinary epidermal cells. It is thus possible that the thread-like base bends off into the nerve-fibre layer; in other words, that the cells are continued into nerve fibres at their bases. On the other hand, it must be borne in mind that the nerve-fibre layer underneath these pits is much thinner than in the surrounding epidermis.

About the nature of these pits with their peculiar cells nothing definite can be said. They give one the impression of being nervous or sensory and remind one of the epidermal sensory organs (lateral line) of fishes. If they are sensory organs their position is rather peculiar. They are found in the branchio-genital and liver regions, thus far away from the proboscis, which with its abundant nerve supply is the most sensitive part of the body, and also far behind the mouth. Further, they are situated in a long row next to the ventral mid-line, so that they are far removed from the branchial pores on the dorsal side, while in the genital region there are no branchial pores at all. Therefore these organs cannot be concerned with the food or with the water for respiration. Their ventral position precludes the possibility that they are organs for light perception.

There is no external circular muscle layer in the trunk. The layer of longitudinal muscle fibres is slightly thicker than the epidermis, except along the medial side of the genital wings, where this layer is distinctly thinner than the epidermis (fig. 36). Also in the dorso-median part, *i.e.* between the two branchial grooves, this layer decreases in thickness near the end of the branchial region (fig. 37). In the genital region the longitudinal muscle layer of the dorsal side is much thinner than that of the ventral side (fig. 42). As in other Ptychoderidae, with the exception of *Gl. mortenseni*, there are no internal circular muscle fibres.

Both the dorsal and the ventral mesentery show many interruptions through which the right and left coelomic cavities intercommunicate (figs. 37 and 40).
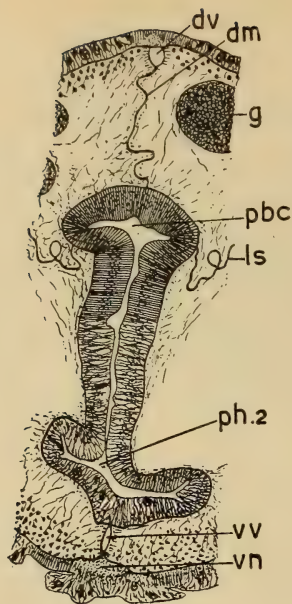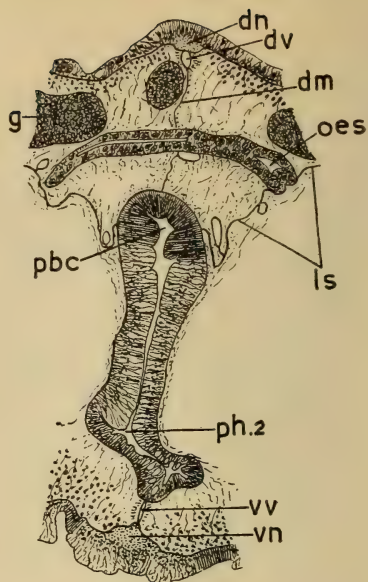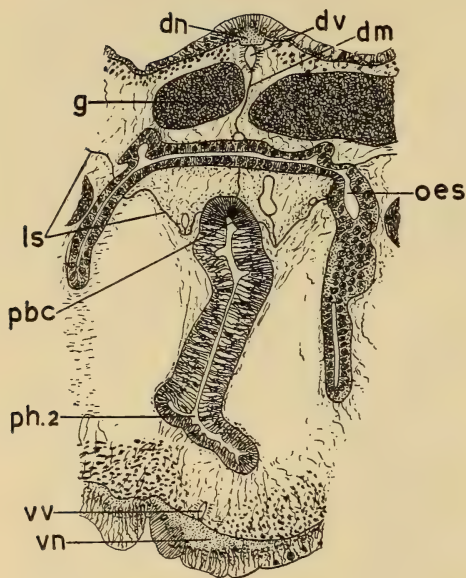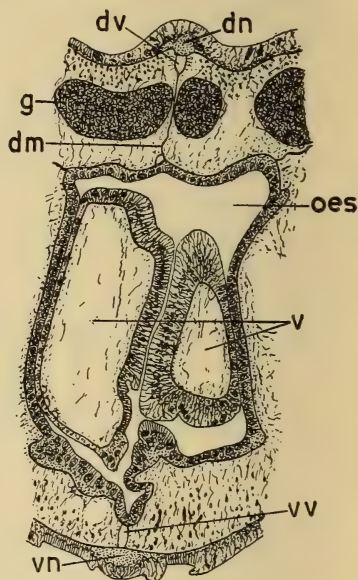
FIGS. 38–41.—*Glossobalanus alatus* n. sp.   38. Cross-section just posterior to the last gill slit.   39. Cross-section of the median part of the trunk a few sections caudal to that of fig. 38.   40. The same, a few sections caudal to that of fig. 39.   41. The same, a few sections caudal to that of fig. 42.   All × 36.

dm, dorsal mesentery.   *dn*, dorsal nerve cord.   *dv*, dorsal blood-vessel.   *g*, gonad. *ls*, lateral septum.   *oes* (figs. 39, 40, 42), anterior blind-sac of oesophagus.   *oes* (fig. 41), oesophagus.   *pbc*, postbranchial canal.   *ph2*, digestive part of pharynx.   *v*, the two valves containing blind-sacs of the coelom and projecting backwards into the oesophagus.   *vn*, ventral nerve cord.   *vv*, ventral blood-vessel.

The dorsal, branchial part of the pharynx is wider than the ventral, digestive part. The limiting ridges, separating these two parts, are not very pronounced.

The wall of the ventral pharynx is thinner than that of the buccal cavity. There are many vacuoles in this epithelium, but they are confined to the outer part of the cells facing the lumen (fig. 37). The nuclei are found in the basal part of the epithelium. Many small granular glandular cells occur near the surface, and long thin glandular cells with a homogeneous, darkly stained substance extend through the whole thickness of the epithelium of the ventral pharynx.

The number of gills is 24 on each side in specimen 3. This is a very small number, corresponding with the shortness of the branchial region. But even in *Gl. ruficollis*, which has an equally short branchial region, the number of gills is 36. The exact number of gills in specimen 1 could not be counted, owing to some irregularities. Several septa had bifurcated, and on the left side there were more of these irregular septa than on the right side.

The number of synapticula is 9.

In specimen 3 and on the left side of the body of specimen 1 the first and second branchial pores were completely separated from each other; on the right side of specimen 1, on the other hand, the two pores had fused to form a single opening, and the epithelium of the collar canal extended to the opening of the second gill.

The epithelium on the back of the tongues is high, that on the back of the septa low; conseqently the tongues protrude farther into the lumen of the pharynx than the septa. There are many glandular cells on the back of the tongues, and similar cells are found also on the epibranchial ridge. Hill found in *Gl. hedleyi* numbers of gland cells occurring in the thin walls of the branchial sacs. Similar cells are also present in the same epithelium of *Gl. alatus*; they have a similar appearance to the granular cells of the epidermis.

The postbranchial canal is very large in this species. It has, as is usual in the genus *Glossobalanus*, a dorsal blind-sac extending forwards above the last gills (fig. 37). As it extends to the level of the fourth last branchial pore it is very deep, even for a species of *Glossobalanus*. As in *Gl. hedleyi* and *Gl. elongatus*, the epithelium of this blind-sac, as well as that of the whole postbranchial canal, does not show irregular folds, but it is very high, consisting of regular, thin, long cells, and by its darker stain it contrasts well with the epithelium of the pharynx (fig. 38). Immediately behind the last

branchial pore the postbranchial canal is connected with the ventral pharynx by a narrow vertical slit (fig. 38). The whole of the intestine is here laterally strongly compressed with a dorsal wider part, which
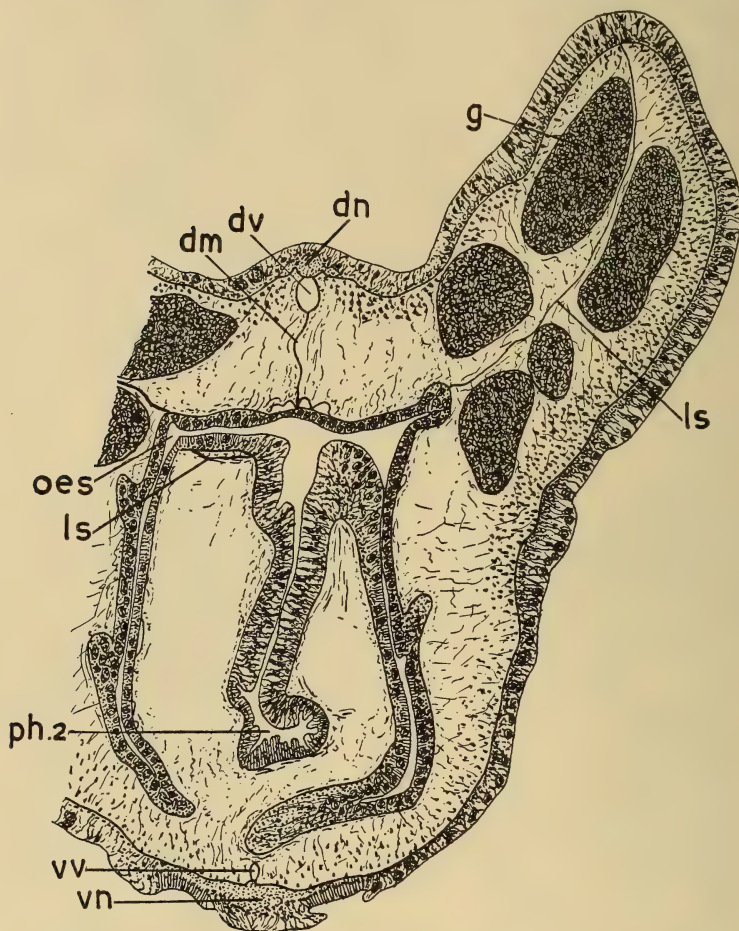
Fig. 42.—*Glossobalanus alatus* n. sp. Cross-section of the trunk a few sections behind that of fig. 40. × 45. For lettering see preceding figure.

is the postbranchial canal, and an even wider ventral part. The latter is the ventral pharynx and not the oesophagus, as is clearly shown by the histological character of its wall. In specimen 1 the connection of this part of the intestine with the oesophagus is quite different from the usual condition in *Glossobalanus* that is also found in specimen 3. In the majority of the Ptychoderidae the ventral

pharynx is directly continuous with the oesophagus; the ventro-lateral corners of the oesophagus then turn upwards, and, reaching the level of the postbranchial canal, they again turn inwards and connect with the postbranchial canal, which here terminates. In this way two valves, containing an extension of the coelomic cavity, are formed and these valves extend for some distance backwards into the oesophagus. In this specimen of *Gl. alatus* there first appears a narrow cavity dorsal to the postbranchial part of the intestine (fig. 39). By the nature of its wall this cavity proves itself to be an anteriorly directed blind-sac of the oesophagus. At first it is quite flat and horizontal, then it turns ventralwards on both sides (fig. 40). It is here that the postbranchial canal terminates, and the main intestinal canal is merely the prolongation of the ventral pharynx. The ventro-lateral corners of the oesophageal blind-sac, having arrived at a level ventral to the main intestinal canal, bend inwards (fig. 42), and at the same time the main canal communicates with the oesophageal blind-sac in the dorsal mid-line. Somewhat farther on the ventral ends of the oesophageal blind-sac fuse with each other in the mid-line and also with the ventral end of the main canal (fig. 41). In this way two portions of the coelomic cavity are cut off from the rest; they are surrounded by the wall of the intestine, which medially exhibits the features of the wall of the ventral pharynx and laterally of the oesophageal wall. These coelomic blind-pouches extend as in other species for some distance into the oesophagus in the form of a pair of valves that may prevent the food in the oeso-phagus from passing back into the branchial pharynx. The epithelial wall of the oesophagus is very thin and contains many darkly stained glandular cells.

The first genital pore is situated on both sides behind the ninth or tenth branchial pore. In specimen 1 this first pore is on one side only, the opening of a short gonaduct, there being no real gonad. There are more of these pores without gonads farther back, as well as immature gonads that have not yet reached the full size. This may be an indication that the number of gonads is still increasing in this specimen. The gonopores are situated exactly on the edge of the genital wings (fig. 36), so that there is no empty, sterile part of the genital wings as in *Gl. marginatus*. In correspondence with the broad genital wings, the gonads are very elongated, reaching to about the middle of the branchial bars. In general they have the form of long sacs, the inferior part of which is broadest and shows irregular protrusions. The gonad can also be branched, especially

in the posterior part of the branchial and the anterior part of the genital regions.

The lateral septum reaches far into the branchial region, beginning even in front of the fifth branchial pore. Nevertheless, the dorsal coelomic cavity remains devoid of gonads for a long distance, the first dorsal branch of a gonad being found at about the level of the 20th branchial pore. There is no doubt, however, that dorsal gonads are found in the branchial region (fig. 37).

As in nearly all species of *Glossobalanus*, there is a ciliated groove on the left side of the intestinal wall only. This groove begins in the genital region, but it cannot extend far into this region. The specimen (fig. 22) must have been broken off at the hind end of the genital region, because the available part of the pleura is 25 mm. long, and in the other somewhat larger specimen, broken off behind the liver region, the pleura are 28 mm. long; also the gonads are very small near the end of the series of sections.

Diagnosis of Glossobalanus alatus.

Genital pleura very broad, like wings, beginning shortly behind the collar, reaching their maximum width in the hinder end of the branchial region, decreasing hardly in size, and disappearing well in front of the liver region. Branchial region very short, $\frac{1}{10}$ the total length of the thorax. In the smaller specimen the proboscis is 1·7 mm. long, the collar about 2 mm., the branchial region 2·5 mm., and the branchio-genital region 25 mm. In the larger specimen the proboscis is 2 mm. long and 3 mm. wide, the collar has a length of 3 mm. and a greatest width of 3·8 mm., the branchial region is 3·3 mm. long, the genital pleura 28 mm., the transitional region 6·5 mm., and the liver region 3·7 mm. The liver region is well delimited both at its anterior and its posterior ends; all liver saccules, 14 of which are visible externally, are approximately of equal size.

Proboscis.—Ventral proboscis septum very short, connecting with the epidermis at the level of the ventro-lateral blind-pouches of the stomochord. Ventral proboscis blind-sacs deep, extending between body and keel of skeleton. Only left dorsal coelomic sac connected with a median end-sac. Proboscis pore median. Lumen of stomochord continuous throughout; two ventro-lateral blind-pouches in stomochord; they may be connected with the main lumen and inter-communicating, or there are at least indications of these connections. Small dorso-lateral blind-pouches of stomochord may be present with common opening into main lumen. Glomerulus surrounds the

anterior ends of pericardium and stomochord; farther caudally the glomerulus covers the pericardium to a greater extent than the stomochord. Keel of skeleton separated from the primary body nearly over its whole length; keel begins on U-shaped anterior part of skeleton, increases suddenly in size, extends nearly over whole length of body, and ends rather abruptly; crura short, surrounding about half the buccal cavity.

Collar.—Second and third epidermal zones of collar with much higher epithelium than the first, fourth, and fifth zones. Dorsal mesentery complete from first or second dorsal nerve root to end of collar. Ventral mesentery only present in extreme caudal end of collar. Collar canals with dorsal fold only. Perihaemal cavities begin at the level of the proboscis pore; their anterior ends adjacent to each other; the mesentery between them entire over whole length. Medullary cord with continuous central canal and 2–4 dorsal roots; no anterior epidermal pouch.

Trunk.—Ventro- and dorso-median grooves present only in anterior end of branchial region; branchial grooves deep. A row of small epidermal pits with an epithelium quite different from that of the epidermis, at both sides of ventro-median line over whole length of trunk. No external nor internal circular musculature in the trunk. Dorsal and ventral mesenteries often interrupted. Dorsal pharynx wider than ventral; limiting ridges feebly developed. About 24 gills and 9 synapticula; first and second branchial pores may be fused. Post-branchial canal with large anterior blind-sac. First genital pore behind the ninth or tenth branchial pore; gono-pores on edge of genital wings; no sterile part in genital wings; gonads large, branched, extending to middle of branchial bars. Lateral septum begins near fifth branchial pore; dorsal gonads present in posterior end of branchial region. Ciliated groove on left side of alimentary canal only, extending slightly into the genital region.

Two New Species of *Balanoglossus*: *B. hydrocephalus* and *B. studiosorum*.

Occurrence.

Balanoglossus is most abundant on the muddy flats along the western side of Inyack Island. Part of these flats is formed by sand mixed with mud; another part, however, in front of which extends a coral reef, contains a great amount of coral debris, which

makes digging with a spade there impossible. By digging in the sandy mud a fair number of specimens was procured, but not one that was quite intact. When, however, a place was found in the coral gravel, where, judging from the castings, *Balanoglossus* was most abundant, digging was also attempted there, although most of it had to be done with the hands. We started to make a circular furrow about a foot deep. Scooping the water from this furrow made the central part more or less dry, so that it was less easy for the animals to escape, and also one could see where one was digging. From this furrow we proceeded to remove the gravel towards the centre; undermining proved to be better than digging from the top layer. In this way we collected quite a number of entire specimens. By following this same method some entire specimens were also collected in the sandy mud later on. In one respect one has to exercise care when digging out the specimens. When the abdominal part is seen first, it is better to leave it alone, even when it disappears in the sand or the gravel, because, when touched, this part of the body invariably breaks off. But when the head end is seen first, it is quite safe to take it in the hand and to dig out the rest of the body carefully. Once the hepatic region has been exposed in this way, one can slowly pull out the whole abdominal region from the burrow without breaking it. This was also observed by Stiasny in *B. clavigerus.*

It was at once clear that the *Balanoglossus* from the gravel was different from that living in the sand. I will describe in the following pages the *Balanoglossus* from the gravel, the proboscis of which is very large, under the name of *B. hydrocephalus*, and as my students helped me so much in collecting this material I wish to name the species from the sandy mud *Balanoglossus studiosorum.*

The existence in gravel or in sandy mud is not the only difference in habitat between these two species. Though the flats, where they live, are nearly level, so that there is hardly any difference in length of exposure at low tide, *B. hydrocephalus* occurs most abundantly near the shore, and in certain patches a great number of specimens are crowded together; in an area of about half a square metre we found 21 specimens. On the other hand, *B. studiosorum* lives in the mud at some distance from the shore, and the specimens are scattered and isolated from each other, so that by adopting the procedure described above only one specimen was procured at a time.

It is possible that a third species of *Balanoglossus* lives on these flats. Judging from its castings, which are heaps of coiled mud
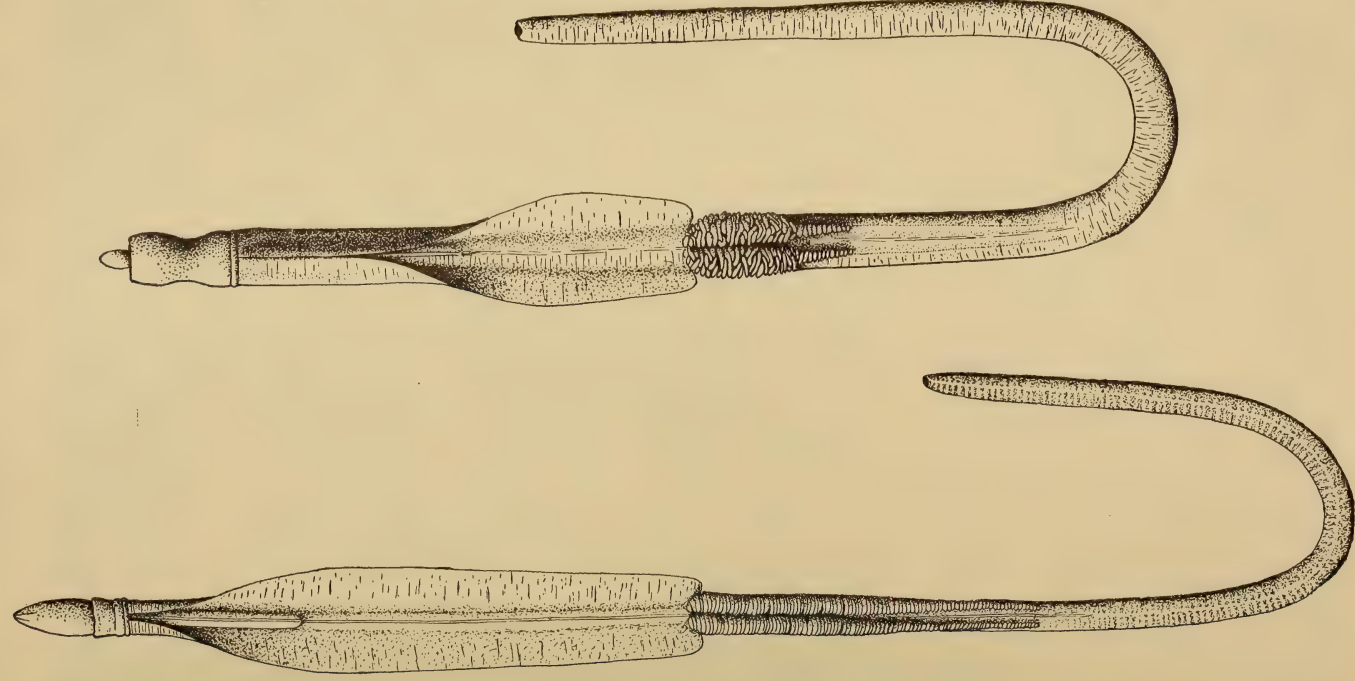
FIG. 43.—*Balanoglossus hydrocephalus* n. sp. (left) and *Balanoglossus studiosorum* n. sp. (right). Both in dorsal view and about two-thirds of nat. size.

about 10 cm. high, this species must be one of great dimensions. Though I tried more than once, I did not succeed in procuring even a small piece of a specimen. Evidently they burrow very deep down into the mud, over two feet at least, and they have disappeared before one is able to dig a furrow as deep as that.

External Features.

Though the two species, *B. hydrocephalus* and *B. studiosorum,* occur very near each other and are of about equal size, they are easily distinguished from each other. On the whole, *B. hydrocephalus* is the more slender and *B. studiosorum* the stouter of the two. The most obvious difference is shown by the relation between the proboscis and the collar. There are some species of *Balanoglossus* (e.g. *B. clavigerus*) in which the proboscis is well developed and longer than the collar, the latter is then about as long as broad. In other species (e.g. *B. carnosus*) the proboscis is greatly reduced in size and protrudes hardly beyond the elongated collar. The two species from Inyack exhibit this difference in a very marked way. *B. hydrocephalus* has a very large proboscis; in the living animal it can easily reach a length of over 2 cm., though it changes its form and thereby its length continually; it has a larger proboscis than any other known species of *Balanoglossus*. But the collar is short; it is at most as long as it is broad. On the other hand, in *B. studiosorum* the proboscis is greatly reduced in size, it never protrudes more than a few millimetres out of the collar, and often in the living as well as in the preserved animal it is quite retracted into the collar. But the collar is very elongated, it reaches a length of about 2 cm., and is about twice as long as it is broad.

This difference in size of proboscis and collar is correlated with a difference in the locomotion of the animals. On the whole *B. studiosorum* is a more sluggish, *B. hydrocephalus* a more active animal. All parts of the body and especially the abdominal region can contract considerably, and though they may help in it, yet the proboscis and collar are the principal organs of locomotion in Enteropneusta. But in *B. studiosorum* the small proboscis can hardly be an organ for locomotion. It is constantly protruded from and retracted into the collar and it moves in all directions. It may help to loosen the sand, but its principal function seems to be that of a sensory organ, somewhat similar to the tongue of a snake. Locomotion is effected especially by the collar, and it is more likely that the animal eats rather than bores its way through the sand. In *B. hydrocephalus*

the condition is quite different. In this animal the proboscis is an active and powerful boring organ. Waves of contraction start at the top and move from there backwards, but the base of the proboscis maintains about the same diameter, as the waves do not quite reach the base. Each wave takes from 5 to 9 seconds to travel over the proboscis, and 9 to 10 movements are made per minute (fig. 44). If the animal is very active, several waves are present at the same time and each wave needs only 3 seconds to cover the length of the proboscis; up to 26 contractions were then counted in one minute (fig. 45).

The branchio-genital region also exhibits some characters which help in the differentiation of the two species. In *B. hydrocephalus* the hind end of the branchial basket is clearly marked by a depression, but in *B. studiosorum* it is very difficult, in some specimens even impossible, to demarcate the branchial from the genital region. In both species the genital wings start immediately behind the collar; anteriorly the wings are fused with the posterior rim of the collar. In *B. studiosorum* the wings in this region already attain a considerable breadth, so that their free edges touch each other in the mid-line, and as a rule they are bent over the dorsal side of the body. Over their whole extent a canal, which is open only at the caudal end of the wings, is thus formed between them and the body. When spread out it appears that the wings increase rapidly in breadth behind the collar and that they keep the same breadth over the greater part of their length; only near their posterior end they become narrower. In *B. hydrocephalus*, on the other hand, the wings are not so broad near their anterior end, so that there is a gap between their free edges. When the wings are bent over the dorsal surface of the body, their edges touch each other except just behind the collar, where a small triangular opening is left. But mostly the wings are spread out in the living animal, and if not, they are easily spread out, which is not the case in *B. studiosorum*. In *B. hydrocephalus* the wings reach their maximum breadth near the hind end of the branchial region and from there they decrease gradually. In one respect the two species correspond, and that is that the wings end abruptly just at the beginning of the liver region. They do not extend into that region as in *B. clavigerus*, nor is there a transitional region between the genital and liver regions as in *B. carnosus*.

In regard to the liver region there is a great difference between these two species of *Balanoglossus*. In *B. hydrocephalus* we find much the same arrangement of the liver saccules as in *B. carnosus*. The liver region is here very long; the anterior end is well marked off;

a few smaller saccules are found here that rapidly increase in size and then the saccules reach their maximum breadth. They form a very regular row at each side of the dorsal nerve cord and each saccule extends over the whole breadth of the row. In the first half of the
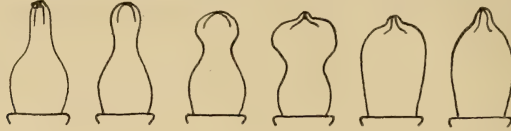
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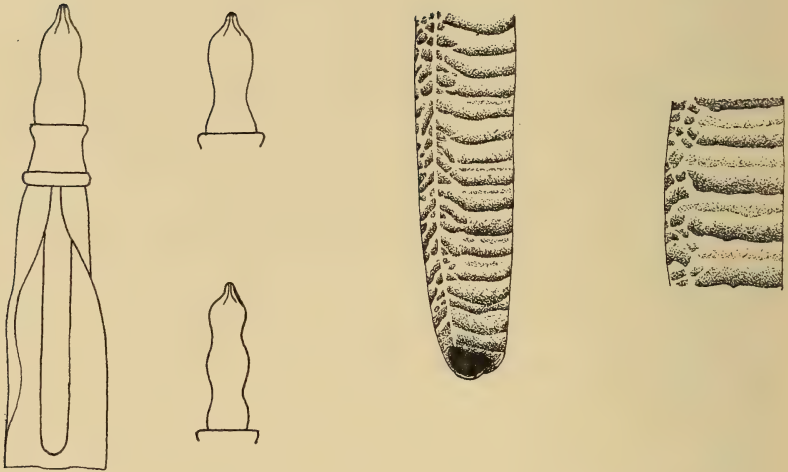
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Figs. 44–46.—*Balanoglossus hydrocephalus* n. sp. 44. Successive stages of contraction of the proboscis of a slowly moving animal. 45. Forms of the proboscis of a quickly moving animal. 46. The caudal region and part of the abdominal region. × 4·5.

liver region the saccules hardly decrease in size, but then they gradually become smaller. At the posterior end of the row they become so small that in many specimens it is hardly possible to determine the posterior end of the liver region, especially as the row of saccules are often continued into two dark lines that extend some distance into the abdominal region. In *B. clavigerus* the smaller saccules near the

posterior end of the rows are situated at some distance from each other, which distance increases towards the posterior end. This is not so in *B. hydrocephalus*; all saccules over the whole extent of the region are closely applied to each other; only when the animal is stretched out very much the saccules may be slightly separated from each other. The number of saccules is very great; there may be about 200 of them.

The liver region is quite different in *B. studiosorum*, where it is short and compact. Each row consists of a number of irregularly arranged saccules that on the surface at least hardly ever extend over the whole breadth of the row, most saccules being considerably narrower. The anterior end of the rows is well defined, the posterior end may be so. But often the broad row of irregular saccules comes to a rather sudden end and is then continued into a short row of much smaller but regularly arranged saccules that gradually decrease in size towards the posterior end.

Like the whole body, the abdominal region of *B. hydrocephalus* is more slender than that of *B. studiosorum*. A separate caudal region could not be discerned in either species.

On the whole the colour of *B. studiosorum* is dull and uniform. The proboscis is pale yellow and the collar dull yellow. The genital wings are yellow shading into orange-brown. The anterior part of liver region is more of a bright orange-brown, and the larger posterior part greenish brown. The abdominal region is colourless; faint transverse striae may be seen here, owing to the presence of the usual glandular ridges, which do not show a regular arrangement.

On the other hand, *B. hydrocephalus* is vividly coloured, and a great variation in the colour was found in different specimens. The proboscis is always yellow; the collar can be yellow like the proboscis or more yellow-brown. In the branchio-genital region the body itself shows a brown colour and the genital wings are often bright brick-red; they may, however, be dark or light brown, or their anterior part is dark brown and their posterior part gradually becomes lighter, hardly differing from the colour of the collar. The anterior part of the liver region, about 1 cm. long, is dark brown, almost black; the rest of the liver saccules is greyish brown. The abdominal region shows a very characteristic colour scheme, by which it is possible to recognise even small detached pieces of the abdominal region as belonging to this species (fig. 46). On a greyish-yellow background occur regular rings of a dark brown to black pigment, which are only broken up into small dots near the mid-dorsal line.

In many places it can be seen that darker rings alternate with lighter ones, and the darker rings show a very well-defined hind limit but they fade away anteriorly.

The dimensions of the various body parts in Enteropneusta are always somewhat dubious, because during life these animals are capable of considerable expansion and contraction. Furthermore, during fixation the regions of the body contract in varying degrees. The proboscis and the abdominal region are especially liable to great extension and contraction, and as the relative lengths of the proboscis and collar are of great importance, and the length of the long abdominal region determines the total length of the animal to a great extent, one has always to bear in mind that these dimensions have to be taken *cum grano salis.* When one pulls an animal out of its burrow in the way described above, the abdomen may be stretched to a length of 20 cm., and when the contents of the intestine are emptied this same abdomen may not be longer than 4 cm. The proboscis of *B. hydrocephalus,* when fully extended, may be well over 2 cm. long; on the other hand, it can be contracted to 5 mm.

The following are the dimensions in mm. of some living animals:—

Proboscis.	Collar.	Branchial region.	Genital region.	Liver region.	Abdominal region.
Balanoglossus hydrocephalus.					
12	7	22	38	35	—
12	5	15	20	20	30
15	6	30	40	35	50
15	7	40	30	35	30
15	6	30	40	30	—
Balanoglossus studiosorum.					
3	20	160		10	70
5	18	132		10	—

These animals were measured the day after they had been collected and when they were already partly anaesthetised; in all the intestinal contents were emptied and therefore the abdomen is contracted, but by pulling it carefully it can easily be extended to a much greater length. Also, the proboscis is shorter than it usually is. The normal length of living specimens of both species is about 30 cm.

If one wants fully extended fixed specimens, one has to kill the animals very carefully. I anaesthetised the animals, but did not dare to go so far that they did not show any movements at all, as a dead animal disintegrates and becomes a slimy mass almost at once. Therefore the partly anaesthetised animals were arranged on a dry board so that they had about the natural dimensions.   Now it appears that the most sensitive parts of the body are the tip of the proboscis and the end of the tail.   As soon as the preserving fluid touches either of these regions they are contracted to their smallest dimensions. Thereby the top of the proboscis is often invaginated to such an extent that there seems to be an opening, which Kowalevsky thought to open into the proboscis coelom.   For this reason I started the fixation in the liver region and proceeded from there towards both ends by adding the fixation fluid drop by drop.   In this way the abdominal region is fixed, and is incapable of further contraction when finally the end of the tail is reached.   With the proboscis, one has to do this even more slowly.   It will not contract when the fluid reaches its base, but even when half the proboscis is killed off, the top will contract immediately when it is reached by the fluid.   Therefore the fixative must penetrate the proboscis from its base only, and then one can obtain specimens with the proboscis well extended.

The dimensions in mm. of some of these preserved animals are as follows:—

Balanoglossus hydrocephalus.

Proboscis.	Collar.	Branchial region.	Genital region.	Hepatic region.	Abdominal region.
13	7	44	82	54	107
11·5	5	59	67	95	137
12	5·5	25	25	44	80
6·5	4·5	20·5	11·5	20·5	10

The maximum distance between the free edges of the outstretched genital wings is 16·5 mm. in the first specimen of this list and 21 mm. in the second, and in the latter the minimum distance that is found near the hind end of the wings is 11 mm.   The proboscis is not flattened, it is more or less conical; its maximum width in the first specimen is 5·5 and in the second 6·5 mm.   The anterior rim of the collar is extended funnel-like round the base of the proboscis.

Behind this extension the collar is often greatly contracted in the living animal as well as in the preserved, so that its diameter here can be considerably less than near its posterior end. For instance, in the first specimen of the above list the diameter of the collar is 7 mm. anteriorly and 8·5 mm. posteriorly.

Balanoglossus studiosorum.

Proboscis.	Collar.	Branchial region.	Genital region.	Hepatic region.	Abdominal region.
0	12	116		16	80
2	8	93		20	120
2	8	215		22	106

The maximum distance between the free edges of the outstretched genital wings in these three specimens is 20, 19, and 21 mm. respectively. But in the third specimen one wing was much broader than the other, the greatest half-breadth being 14 mm. The collar, though somewhat constricted in the middle, has about the same diameter anteriorly as posteriorly; in the second and third specimens of the above list this diameter is 7 and 8 mm. respectively.

In 1908 Gilchrist described a species of *Balanoglossus* under the name of *Ptychodera natalensis* from Durban Bay. Unfortunately Gilchrist gives the external characters of this species only; though these are described rather accurately, they are not sufficient to identify the species beyond doubt, especially as the description is not accompanied by a figure of the animal. Furthermore, Gilchrist's specimens, from which the description was made, cannot be found. At first I expected that one of the species from Inyack would be identical with *Balanoglossus (Ptychodera) natalensis*. It is clear that *B. studiosorum* is certainly different from *B. natalensis*, but *B. hydrocephalus* agrees in some respects with it. According to Gilchrist the proboscis of *B. natalensis* is relatively short and was not observed to vary much in the living animal. It was about 11 mm. in length and in the preserved condition 9·5 mm. Judging from the other dimensions given by Gilchrist, his specimen must have been large for *B. hydrocephalus*, and then the proboscis should have been considerably longer than 11 mm. As previously mentioned, the proboscis can easily reach a length of 2 cm. during life; I have a preserved specimen in

which it is still 19·4 mm. long.   The dimensions of the collar, 6 mm. long and 8·5 mm. broad, agree with those of *B. hydrocephalus.*   In his description of the branchio-genital region Gilchrist obviously makes a mistake.   He says that this region was broken up into four pieces, respectively 90, 56, 42, and 16 mm. long, so that the total length of this region was a little over 200 mm.   Having described the first and second fragments, Gilchrist says that the third and smallest fragment includes part of the liver region, that a few hepatic coeca occur in a small part of the pleural (*i.e.* genital) region over a length of about 6 mm., and further, "the fourth fragment was 42 mm. in length, and was covered with the hepatic coeca."   Therefore it seems that the branchio-genital region was 156 mm. in length and not 200 mm., of which the branchial region measures 45 mm.   The length of the liver region agrees with that of *B. hydrocephalus,* but in *B. natalensis* the hepatic coeca are arranged at first in a single row on each side and are hardly compressed, becoming towards the centre larger, crowded together, and arranged in an irregular mass, and this is different from what is shown by *B. hydrocephalus.*   On the other hand, both species have the pigmented annular rings, which are broken up into isolated patches along the dorsal side, in the abdominal region, and also the coloration of the rest of the body of *B. natalensis* is similar to that of *B. hydrocephalus.*   The only internal character of specific value mentioned by Gilchrist is that there appears to be one nerve root only, and this is not the case in *B. hydrocephalus.*   If everything in Gilchrist's description is taken into consideration, I think that it is better to describe the specimens from Inyack as belonging to another species—*Balanoglossus hydrocephalus.*

Internal Anatomy.

Proboscis.—In *B. hydrocephalus* the nuclei of the epidermal cells are situated in the basal half of the cells (fig. 47), whereas in *B. studiosorum* they form a thick layer in the middle of the epidermis (fig. 50). In the latter species all ordinary epidermis cells seem to contain mucus that fills the peripheral and basal parts of the cells and is discharged at the surface in great quantity.   On the other hand, in *B. hydrocephalus* special glandular cells occur in between the undifferentiated epithelial cells; these glandular cells are very narrow, extend over the greater part of the thickness of the epidermis, though they do not reach the nerve layer; they stain very darkly with haematoxylin.   In both species small glandular eosinophil cells are

found; they occur especially in the outer part of the epidermis; towards the nerve layer they become less numerous; the glandular

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Fig. 47.—*Balanoglossus hydrocephalus* n. sp.   Cross-section of a part of the proboscis.

Fig. 48.—*Balanoglossus studiosorum* n. sp.   The same.
Both × 38.

c, centre of proboscis. *cm,* circular musculature. *ct,* connective tissue. *epd,* epidermis. *lm,* longitudinal musculature. *nl,* nerve-fibre layer. *pc,* proboscis cavity.

part of these cells is almost round or oval, and they often show a narrow tube-like extension to the surface. In *B. studiosorum* a dense layer of nuclei, apparently the nuclei of the nerve cells, is

situated just outside the nerve-fibre layer; in *B. hydrocephalus* there are not so many nerve-cell nuclei. The layer of nerve fibres has about the same thickness in both species.

In *B. studiosorum* the layer of circular muscles is very thin, it is not thicker than about one-third of the nerve-fibre layer (fig. 48). In *B. hydrocephalus*, on the other hand, the circular musculature is very conspicuous and reaches to a thickness equal to that of the nerve-fibre layer (fig. 49). A sphincter at the base of the proboscis, such as occurs in *B. clavigerus*, is missing in both species.

The longitudinal muscle fibres are especially numerous in the peripheral part of the proboscis in *B. hydrocephalus*; towards the centre they become less concentrated and show a tendency to arrange themselves in small bundles (fig. 47). They are split up radially, as is usual in the Ptychoderidae, and it is clearly shown that the radial cracks are pre-established. Strands of fine connective-tissue fibres intersect the longitudinal musculature in a radial direction, and it is along these strands that the radial cracks occur. This was clearly shown in one specimen of *B. hydrocephalus*, in which the muscles had not contracted so much as they usually do, so that some radial strands of connective tissue were quite intact, others were torn up over a short distance, and some split up over their whole length.

In *B. studiosorum* the longitudinal muscle fibres show quite a different arrangement, and, moreover, they are far less numerous (fig. 48). They are concentrated along the central core of connective tissue, and hardly any fibres occur in the peripheral part of the proboscis. They are not arranged in little bundles, and the radial cracks are very irregular and do not reach the central connective tissue. Also, the fibres apparently do not follow an almost straight course; most of them are cut across in the cross-sections and run therefore in fronto-caudal direction, but others are seen following a radial and even a tangential course. Strands of fibres overbridge the radial cracks; these, of course, were running nearly radially, but have been pulled aside by the occurrence of the cracks.

A dorso-ventral muscle plate is well developed in *B. hydrocephalus*, but it does not extend in front of the central proboscis organs (fig. 49). On the other hand, in *B. studiosorum* such a muscle plate is almost entirely absent; only a few muscle fibres are seen running along the sides of the pericardium.

On the whole, the proboscis musculature is very strong in *B. hydrocephalus* and poorly developed in *B. studiosorum*, as was anticipated.

A very large central proboscis cavity was found in *B. hydrocephalus* (fig. 47). It extends over the posterior two-thirds of the proboscis and has a regular conical form with the point directed anteriorly. In front of this cavity and also surrounding its anterior part, where the longitudinal muscles are not so strongly developed as in the posterior part of the proboscis, a very loose connective tissue is found; more posteriorly the muscle fibres extend to the cavity. No cells could be seen surrounding the cavity, only a regular layer of connective-tissue fibres. I have never seen so large a proboscis cavity in any other species of *Balanoglossus*. On the other hand, in *B. studiosorum* there is practically no proboscis cavity at all, an open space being found in the ventral blind-sac and near the proboscis pore only (fig. 48). A central core of connective tissue extends over the whole length of the proboscis, and the glomerulus and other proboscis organs are quite enveloped by this tissue.

The ventral proboscis septum of *B. hydrocephalus* starts somewhat in front of the ventral dilation of the stomochord, and except for some perforations in its anterior part it is complete to the end of the ventral coelomic blind-sacs, which therefore are entirely separated from each other. The anterior part of the septum lying in the ventro-dorsal muscle-plate is very thin, and the ventro-median proboscis vessel running along its anterior edge is very inconspicuous. The posterior part of the septum is very thick and swollen, because many cells have penetrated into the septum, giving it a spongy appearance (fig. 50). The ventral coelomic blind-sacs are rather narrow, and end at the level of the caudal end of the ventral dilation of the stomochord. These blind-sacs have not the regular epithelial wall as in most other species, but are filled with cellular tissue (fig. 50). At their hind end they break up into chondroid tissue, which extends into the skeleton and separates the end-plate from the keel of the skeleton in much the same way as in *B. clavigerus* according to Spengel's description.

In *B. studiosorum* the ventral proboscis septum is short; it starts about midway between the top of the stomochord and its ventral dilation, or even farther backwards, and it stops already near the frontal side of the ventral dilation. The ventral proboscis artery, which should run through the septum, was not visible. So the ventral coelomic blind-sacs have fused to a single cavity over a great extent (fig. 58). This ventral blind-sac is large compared with other species of *Balanoglossus*, and, as in *Ptychodera*, protrudes at the ventral surface of the proboscis neck without, however, forming a racemose
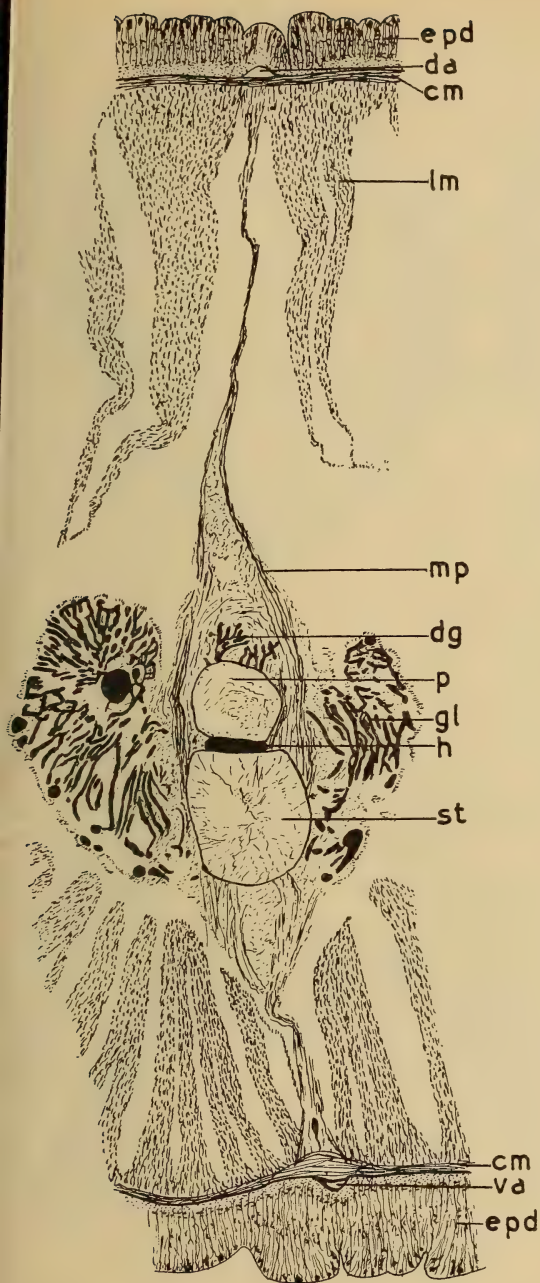
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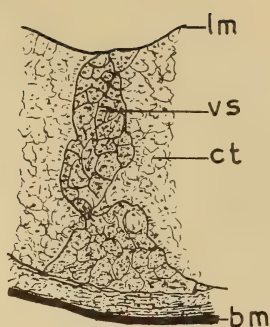
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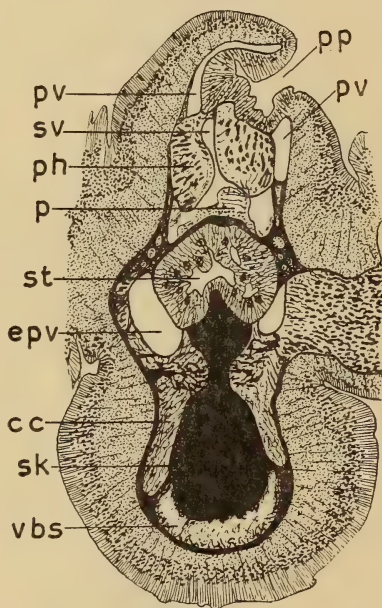
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FIGS. 49–51.—*Balanoglossus hydrocephalus* n. sp. 49. Median part of a cross-section through the proboscis at a level near anterior end of proboscis organs. ×24. 50. Cross-section of the ventral proboscis septum. ×110. 51. Cross-section of the proboscis neck. ×40.

bm, basal membrane of the epidermis. *cc*, collar coelom. *cm*, circular musculature. *ct*, cellular tissue filling the ventral coelomic blind-sacs. *da*, dorsal proboscis artery. *dg*, dorsal glomerulus. *epd*, epidermis. *epv*, efferent proboscis vessel. *gl*, glomerulus. *h*, central blood space. *lm* (fig. 49), longitudinal musculature. *lm* (fig. 50), limiting membrane surrounding the stomochord. *mp*, dorso-ventral muscle-plate. *p*, pericardium. *ph*, perihaemal cavity. *pp*, proboscis pore. *pv*, proboscis veins. *sk*, end-plate of skeleton. *st*, stomochord. *sv*, sinus venosus. *va*, ventral proboscis artery. *vbs*, ventral coelomic blind-sac. *vs*, ventral proboscis septum.

organ.   The sac is almost circular or rectangular in cross-section; at any rate it is as high as broad.   In caudal direction it extends well beyond the end-plate of the skeleton, and there occupies the place of the keel.   It is surrounded here, mostly along its lateral sides, by chondroid tissue, and it ends just where the skeletal keel protrudes farthest, the caudal end making a small indentation in the anterior surface of the keel (fig. 59).   *B. hydrocephalus* differs from *B. studiosorum* in that in the latter species the ventral proboscis blind-sac is lined by a regular layer of cells and shows an open cavity (fig. 58).

As is usually the case, only the left dorsal coelom is connected by way of an end-sac with the proboscis pore in both species.   There is not even the slightest indication of an opening at the right side neither in the end-sac nor in the coelom.   In other species, e.g. *B. stephensoni*, the end-sac has a forward extension on the right side, or, when the opening is on the right side as in *B. capensis*, a forward extension is found on the left side.   In *B. hydrocephalus* the end-sac is rather small and not prolonged beyond the caudal end of the pore. The pore itself is also small and situated on top of a dorso-median or slightly left tubercle (fig. 51).   In *B. studiosorum* the end-sac is long and narrow, corresponding with the form of the pore and the end-sac has a small prolongation beyond the posterior end of the dorso-median pore (fig. 52).

The stomochord of *B. hydrocephalus* is well developed.   Its anterior part is nearly round in cross-section (fig. 49).   At the place of its ventral dilation it becomes much broader, but its dorso-ventral diameter hardly increases.   In the whole body of the stomochord a central lumen is absent;   there are, however, numerous isolated small cavities.   But the ventro-lateral blind-pouches are large, although they are not connected with each other and, of course, neither with the main lumen, as this is not yet present at this level (fig. 53).   There are no dorso-lateral blind-pouches.   The main lumen appears only in the neck of the stomochord, and even here it may be interrupted and irregular in outline, although in one of the specimens it is continuous throughout the neck.   Towards the posterior end, in front of its communication with the buccal cavity, the lumen widens out considerably (fig. 54).   As the crura of the skeleton stand out nearly at right angles, the stomochord communicates with the buccal cavity by a very large opening, which shows a frontally directed narrow median slit between the basal parts of the crura.   The neck of the stomochord has two dorso-median blind-sacs, directed frontally, and
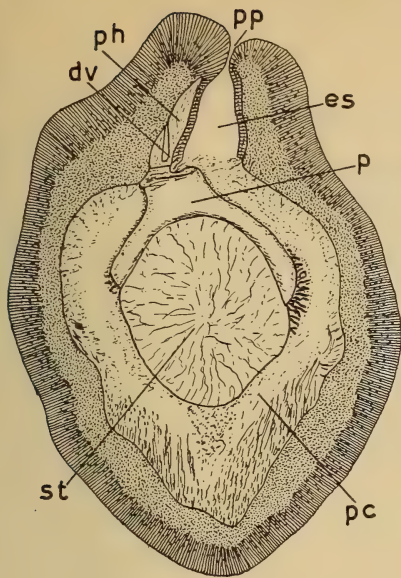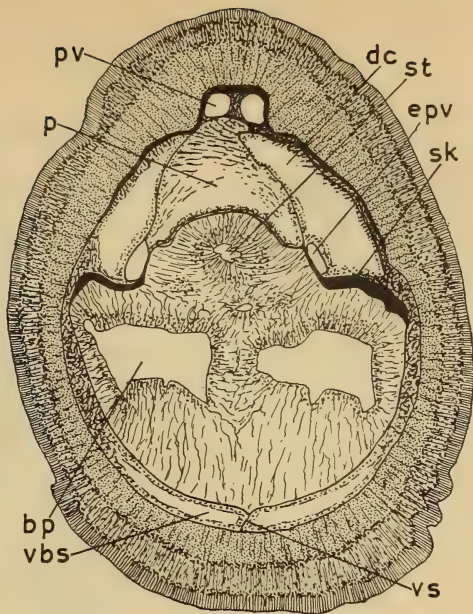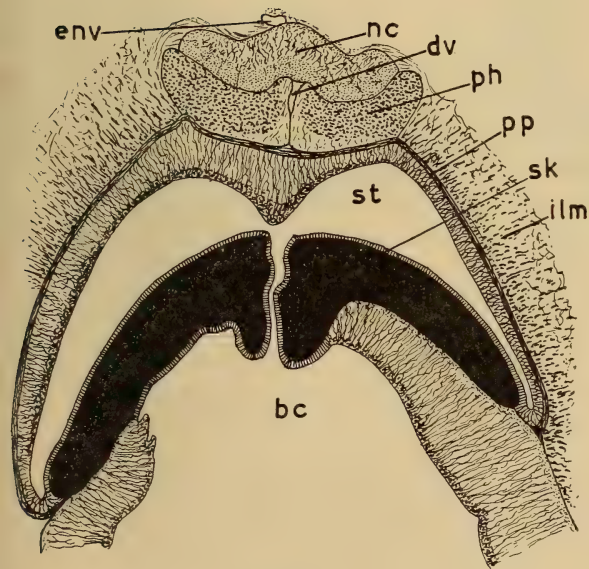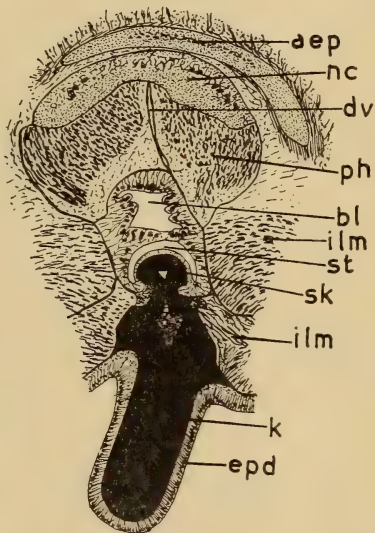
FIG. 52.—*Balanoglossus studiosorum* n. sp.   Cross-section of the proboscis neck.   × 21.

FIGS. 53–55.—*Balanoglossus hydrocephalus* n. sp.   53. Cross-section of the proboscis at level of the blind-pouches of the stomochord.   × 35.   54. Cross-section of the dorso-median part of the collar at the level of the opening of the stomochord into the buccal cavity.   × 21.   55. Cross-section of the dorso-median part of the collar at level where the proboscis neck has fused with the collar.   × 21.

aep, anterior epidermic pouch. *bc*, buccal cavity. *bl*, dorsal blind-sac of the neck of the stomochord. *bp*, ventro-lateral blind-pouch of the stomochord. *dc*, dorsal coelomic cavity. *dv*, dorsal blood-vessel. *env*, epineural vessel. *epd*, epidermis. *epv*, efferent proboscis vessel. *es*, end-sac. *ilm*, internal longitudinal musculature. *k*, keel of skeleton. *nc*, nerve cord. *p*, pericardium. *pc*, proboscis coelom. *ph*, perihaemal cavity. *pp* (fig. 52), proboscis pore. *pp* (fig. 54), peripharyngeal cavity. *pv*, proboscis vein. *sk*, skeleton. *st*, stomochord. *vbs*, ventral coelomic blind-sac. *vs*, ventral septum.

in the epithelium of this part of the stomochord occur many glandular cells of the same type as are found in the wall of the buccal cavity (fig. 55).

In *B. studiosorum* the stomochord is different from that of *B. hydro-cephalus*, and agrees more with that of *B. numeensis* described by Maser, which is also a species with a very small proboscis and a large collar. The anterior part is round (fig. 52), and in the region of the ventral dilation the cross-section of the stomochord is nearly square. Furthermore, there is no main lumen in the body, but small isolated cavities which are surrounded by numerous glandular cells that stain dark blue. The ventro-lateral blind-pouches are small and not connected with each other (fig. 58). There is an indication of dorso-lateral blind-pouches in *B. studiosorum*; in *B. numeensis* these are well developed, but in *B. hydrocephalus*, as previously mentioned, they are quite absent. In *B. studiosorum* there is just a dorso-lateral extension of the stomochord, but a real lumen, as in the ventro-lateral pouches, could not be detected here, only some isolated cavities surrounded by glandular cells being present. The neck of the stomo-chord, like this whole part of the proboscis, is very short. The anterior part of the neck especially is very irregular; outgrowths from the skeleton penetrate into it and may cut it up entirely. In this respect *B. studiosorum* resembles *B. carnosus*, another species in which the proboscis is reduced. The lumen accordingly is here greatly reduced, and it is only in the posterior part of the neck, caudal to the skeletal outgrowths, that a well-developed main lumen appears in the stomo-chord, which opens into the buccal cavity. As the keel of the skeleton protrudes caudally the anterior part of the opening of the stomochord is forked (fig. 59).

The pericardium does not exhibit anything in particular in either species. Only in one specimen of *B. hydrocephalus* is the frontal point of the pericardium pushed in by the muscle plate, with the result that the pericardium extends slightly more in frontal direction lateral to the muscle plate than in the mid-line, thereby forming a pair of short horns. These pericardial horns, however, are not so pronounced as in *Gl. ruficollis* or the Spengeliidae; at the best they are like those of *B. misakiensis*, in which species the top of the pericardium is pushed in in the mid-line by a dorsal extension of the stomochord.

The central blood-space also does not show anything of particular interest; it may extend freely in the anterior part of the pericardium as described by Hill for *B. australiensis*.

The glomerulus is larger in *B. hydrocephalus* than in *B. studiosorum*. Besides that it extends well in front of the pericardium and the stomochord and stops only at the level of the ventro-lateral blind-pouches of the stomochord in the former species, whereas in the latter
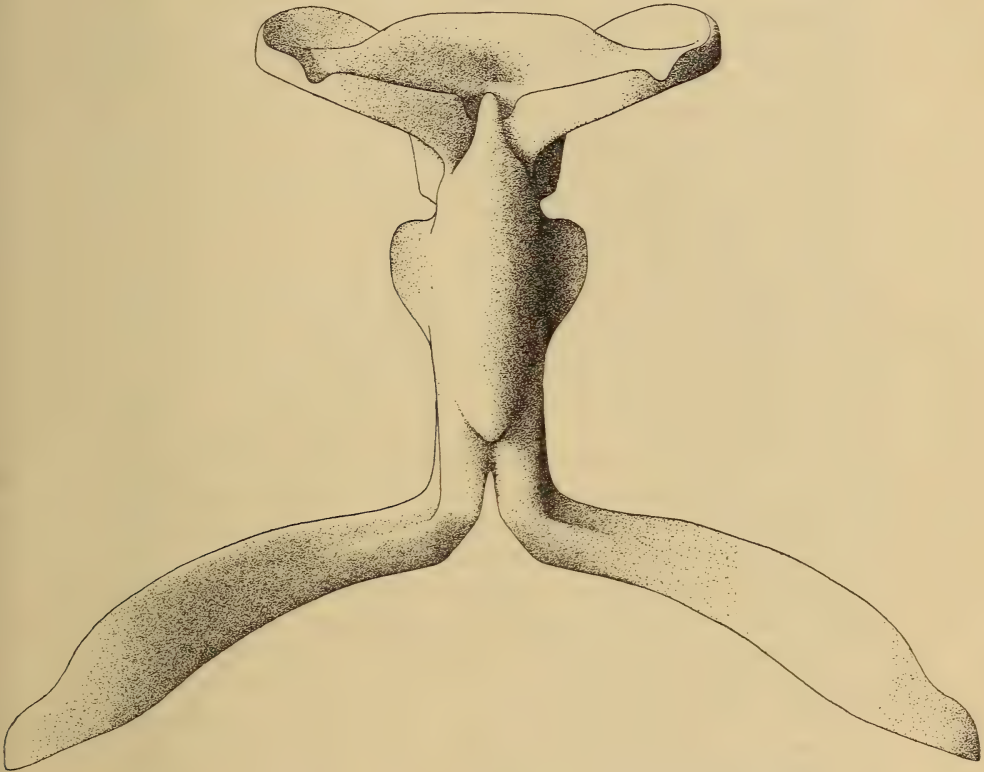

FIG. 56.—*Balanoglossus hydrocephalus* n. sp. Proboscis skeleton from a wax model, ventral side.   × 33.

it does not extend in front of the stomochord and stops in front of the blind-pouches, it is also much broader and its vessels are thicker in *B. hydrocephalus* than in *B. studiosorum* (fig. 49). The function of the glomerulus is not quite known. That it is an excretory organ is beyond doubt, but besides it may help in extending the proboscis by secreting water into the proboscis cavity. Its greater size in *B. hydrocephalus* would then be in correspondence with the size of the proboscis. In this species the two lateral halves of the glomerulus, although extending in front of the pericardium, are separated here

from each other by the fibres of the dorso-ventral muscle plate. Farther caudally the glomerulus covers the lateral walls of the pericardium for the greater part and hardly, if at all, extends on to the sides of the stomochord. But where the fibres of the dorso-ventral muscle plate run along and are fixed to the sides of the pericardium, these fibres separate the glomerulus from the pericardial walls. Besides these two lateral parts, forming the main glomerulus, *B. hydrocephalus* has also a dorsal glomerulus, covering the free dorsal side of the pericardium, and although it is narrow this dorsal glomerulus is very elongate; it is present already at the top of the pericardium and stops only shortly in front of the main glomerulus, where the dorsal edge of the pericardium connects also with the basal membrane of the epidermis (fig. 49).

An interesting point of difference between the two species in question is formed by the skeleton, which supports the stomochord, strengthens the connection between the proboscis and the collar, and serves as a base of attachment for the inner longitudinal musculature of the collar. In correspondence with the size and function of the proboscis, the skeleton is well developed in *B. hydrocephalus*, and in general it does not greatly differ from the form usually found in Enteropneusta (fig. 56). The convex end-plate has a small projecting rim surrounding the ventro-lateral blind-pouches of the stomochord; it also shows a short dorso-median point that projects forwards between the neck and the ventral dilation of the stomochord. Though the outside of the end-plate has the tapering form of a funnel, the inside has not, because the centre is very thick and projects forwards. The body of the skeleton is well developed and shows two pairs of short wings, one at the anterior end and one in the middle. The keel is very large and, covered by the epithelium, it projects far into the mouth-opening (fig. 55). Anteriorly the keel is separated from the end-plate by the ventral proboscis blind-sacs, therefore its anterior end points freely forwards. In caudal direction it decreases gradually in size, nevertheless its posterior end is detached from the body of the skeleton and points freely backwards; this point does not reach the level where the crura separate from each other. The crura at first hardly deviate from the longitudinal direction, only a narrow slit by which the stomochord connects with the buccal cavity being found between them (fig. 54). But then they turn suddenly and stand out from the longitudinal axis nearly at right angles; in one specimen they are even turned forward, but in the other the more lateral parts of the crura bend slightly in caudal direction; neverthe-

less in this specimen the whole of the crura are also situated near the anterior end of the collar.

In *B. studiosorum*, with its small proboscis, the skeleton is correspondingly poorly developed even more than in *B. numeensis*, another species with a small proboscis (fig. 57). The end-plate has an upstanding rim round the ventro- and dorso-lateral extensions of the stomochord and its central part is very convex, with a prominent dorso-median point protruding between the neck of the stomochord and its ventral dilation. But unlike *B. hydrocephalus*, the endplate is rather thin, as its under side is concave and follows more or less its frontal surface. But the whole body of the skeleton is missing, the end-plate being connected dorsally and ventrally directly to the crura; at best the dorsal connection could be considered as a very short secondary body (fig. 58). The ventral connection is very narrow and paired, as there is a large opening at the middle of the ventral side between the end-plate and the keel. This opening extends to the central part of the skeleton and separates here the dorsal from the two ventral connections, and by this opening in the centrum of the skeleton the right and left coelomic cavities of the collar, filled with muscle fibres, communicate with each other; it occurs where the body of the skeleton should be. The keel, otherwise an elongate structure, is very short; it is hardly more than a point that protrudes considerably into the mouth-opening. The anterior surface of this keel is hollowed out and in this hollow is situated the caudal end of the ventral coelomic blind-sac of the proboscis (fig. 59). Being separated from the end-plate, the keel has only a narrow lateral connection with the crura. *B. numeensis* hardly seems to have a proper keel; Maser writes that only "Seitenplatten" are present; these "Seitenplatten" may correspond to the connections between the keel and the crura of *B. studiosorum*. The crura stand out at right angles and are the best developed part of the skeleton, although they are thin. They have a very characteristic form, more or less like two very deep spoons fused in the mid-line and with the handles turned to the lateral sides and extending only into the dorsal wall of the buccal cavity. The plate of the spoons is very broad and its deeply concave surface is facing anteriorly. As aforementioned, the proboscis is connected to the collar about in the middle of the latter, and therefore the crura are situated about in the middle of the length of the collar. The longitudinal musculature is very strong in the anterior half of the collar, and the muscle fibres find a base of attachment in the broad, concave, anterior surface of the crura.
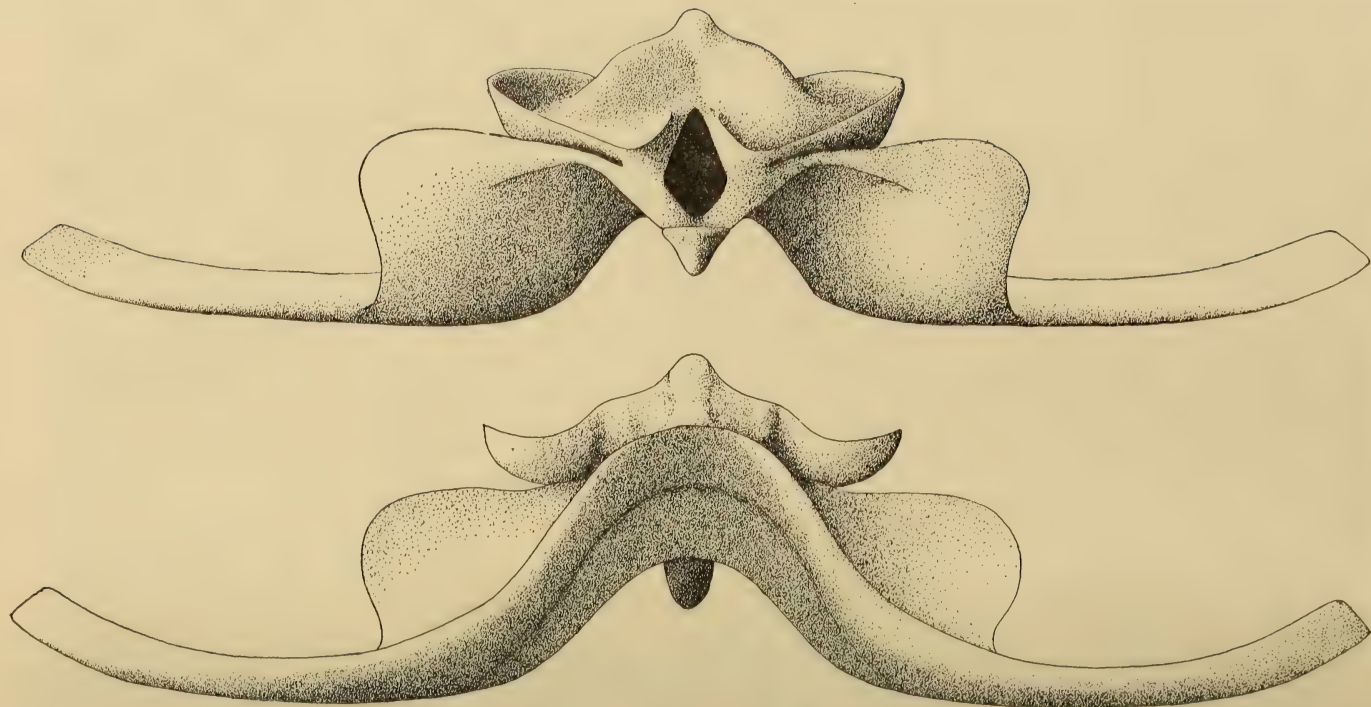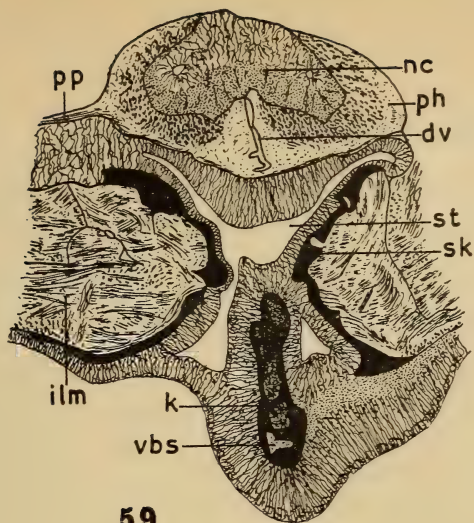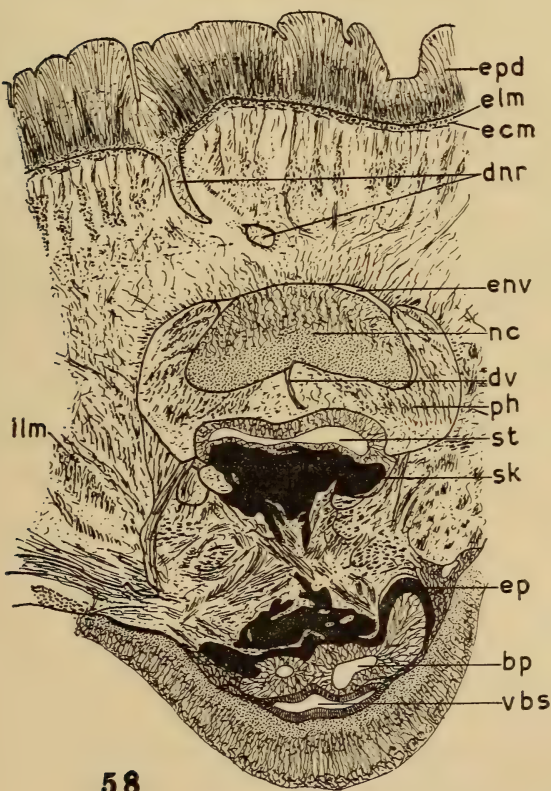
FIG. 57.—*Balanoglossus studiosorum* n. sp.   Proboscis skeleton from a wax model, ventral side (above), dorsal side (below).   ×40.

FIGS. 58, 59.—*Balanoglossus studiosorum* n. sp. 58. Cross-section of the dorso-median part of the collar at level where the proboscis neck has fused with the collar. 59. Cross-section of the dorso-median part of the collar shortly in front of the opening of the stomochord into the buccal cavity. Both × 21.

bp, ventro-lateral blind-pouch of stomochord. *dnr*, dorsal nerve root. *dv*, dorsal vessel. *ecm*, external circular musculature. *elm*, external longitudinal musculature. *env*, epineural vessel. *ep*, end-plate of skeleton. *epd*, epidermis. *ilm*, internal longitudinal musculature. *k*, keel of skeleton. *nc*, nerve cord. *ph*, perihaemal cavities. *pp*, peripharyngeal cavity. *sk* (fig. 58), secondary body of skeleton. *sk* (fig. 59), crus of skeleton. *st*, stomochord. *vbs*, ventral coelomic blind-sac of proboscis.

Collar.—The epidermis of the collar shows the different zones as usually present, but I do not find them well differentiated from each other in either species.

Concerning the musculature of the collar there is a great difference between *B. hydrocephalus* and *B. studiosorum*, as could be expected in accordance with the difference in size and function of this part of the body. In the former species the muscles show the arrangement usually found in the Ptychoderidae. Along the outside of the collar, just under the basal membrane of the epidermis, we find the outer longitudinal muscles, and inside them, at least in the anterior part of the collar, is the layer of the external circular musculature (fig. 60). Both layers, the longitudinal and the circular musculature, are well developed and of about equal thickness in the anterior part of the collar that encloses the proboscis neck; farther caudally the circular musculature disappears altogether and the external longitudinal muscle layer becomes very thin, much thinner than the internal musculature along the wall of the buccal cavity. The latter is, as usual, interrupted along the dorsal side of the collar nerve cord; the muscles of the perihaemal cavities at the ventral side of the nerve cord here supplement the internal longitudinal musculature (fig. 54). Dorsally the internal longitudinal muscle fibres are attached to the proboscis skeleton, and here the collar coelom sends forward two finger-like extensions, filled with longitudinal muscle fibres, that penetrate deeply into the proboscis neck (fig. 51). To the proboscis skeleton are also attached the muscle fibres that spread out along the anterior funnel-like surface of the collar and here form an internal longitudinal muscle layer. This layer is only thin in *B. hydrocephalus*, at most as thick as either the external longitudinal or external circular layer at the same level. An internal circular layer along the anterior surface of the collar could not be found. The radial muscle fibres, traversing the coelomic cavity between the outside of the collar and the funnel-like anterior surface or, more posteriorly, between the epidermis and the wall of the buccal cavity, are not very numerous and rather thin in *B. hydrocephalus*.

In *B. studiosorum* the anterior part of the collar in front of the connection between the proboscis and the collar has not the form of a funnel but more that of a hollow cylinder surrounding the proboscis. The external longitudinal and circular muscle layers are slightly stronger than in *B. hydrocephalus* (fig. 61). The internal longitudinal musculature, however, is very strong, not only in the posterior part of the collar along the buccal cavity but also in the anterior cylinder-

like part.   Here the internal longitudinal musculature forms a layer at least as thick as the external longitudinal and circular together. Usually in Enteropneusta the internal longitudinal muscle fibres stop at the vascular fold surrounding the buccal cavity, and the internal fibres along the anterior funnel-like wall of the collar form another set, spreading out from the proboscis skeleton.   But in *B. studiosorum* the internal longitudinal musculature does not stop at the vascular fold, but is continuous with the layer along the inside of the cylinder. Only at the dorsal side the broad thin crura of the skeleton intersect this muscle layer, and the fibres are attached to both sides of the crura.   Finger-like extensions of the collar coelom into the proboscis neck, in which otherwise a great part of the muscle fibres arise, are absent in *B. studiosorum*, not only because there is no real proboscis neck, but also because of the continuity of the internal longitudinal musculature in front of and behind the attachment of the proboscis. As in *B. numeensis* and *B. aurantiacus*, longitudinal muscle fibres as well as radial fibres are also found dorsally to the nerve cord, though the layer here is not very thick.   The radial musculature between the inner and the outer wall of the cylinder is much stronger in *B. studiosorum* than in *B. hydrocephalus*.   Not only are the fibres thicker but they are also more numerous.   In the peripheral half of their course they form irregular bundles and there are open spaces between the bundles, whereas in the central half they run independently and are embedded in connective tissue.   These open spaces, filled with coelomic fluid which probably is just water, apparently make the action of the musculature easier.   In *B. hydrocephalus* the whole coelomic cavity is filled with loose connective tissue (fig. 60).   The action of the radial musculature seems to be antagonistic to both the circular and longitudinal musculature.   Upon contraction of the radial fibres the outer and inner walls of the cylinder approach each other, so that the cylinder either has to become longer or get a greater diameter.   The first will happen if the circular musculature is contracted and the longitudinal relaxed, and the second if the longitudinal is contracted and the circular relaxed.

As in many Ptychoderidae the dorsal mesentery of *B. hydrocephalus* is absent in the anterior part of the collar and starts only at the back of the first dorsal nerve root.   But whereas in one specimen it is complete from there to the end of the collar, in the other sectioned specimen it stops again some way behind the last nerve root and is reduced then to a broader or narrower vascular fold along the dorsal

side of the collar nerve cord, and only over a short distance near the hind end of the collar is it complete again. In this same specimen also the ventral mesentery is represented by a broad vascular fold hanging down from the wall of the buccal cavity, and it is only in the posterior part of the collar that this fold attaches itself to the basal membrane of the epidermis, thereby forming a complete ventral mesentery. In the other specimen the vascular fold extends over a shorter distance and therefore the ventral mesentery is longer.

In *B. studiosorum* the dorsal mesentery is greatly reduced. In the long anterior part of the collar it is absent altogether, neither does it start at the back of one of the dorsal roots. There is a vascular fold, containing the epineural vessel, caudal to the last nerve root, and this vessel gives off a few side branches that run free through the coelomic cavity to the epidermis; only near the posterior end of the collar a dorsal mesentery is present. In the ventral mid-line a broad and complicated vascular fold hangs down from the wall of the buccal cavity, but it connects with the basal membrane of the epidermis only near the posterior end of the collar, thereby establishing a short ventral mesentery.

The tops of the perihaemal cavities, applied to each other, are found at the level or slightly in front of the proboscis pore in *B. hydrocephalus* (fig. 51). Except for some small openings ventral to the dorsal vessel the two cavities are quite separated from each other (fig. 55). In *B. studiosorum* the perihaemal cavities also reach the level of the proboscis pore, but on account of the absence of a proboscis neck, the pore is situated far forwards in respect to the other proboscis organs; the perihaemal cavities extend in frontal direction quite a distance along the dorsal side of the pericardium and therefore well in front of the sinus venosus (fig. 52). There is hardly a sinus venosus in this species; the dorsal blood-vessel of the collar is directly continuous with the dorsal vein of the proboscis that is situated between the anterior ends of the perihaemal cavities and splits up into two dorso-lateral veins in front of these cavities. Near the posterior end of the pericardium this dorso-median vessel gives off two branches that connect along the sides of the pericardium with the central blood space. As in *B. hydrocephalus* the perihaemal cavities of *B. studiosorum* are only connected with each other along a narrow opening at the ventral side of the dorsal vessel (fig. 58).
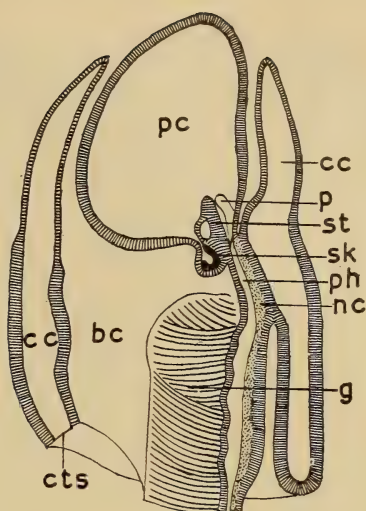
In *B. hydrocephalus* the peripharyngeal cavities, surrounding the buccal cavity, are quite separated from the perihaemal cavities
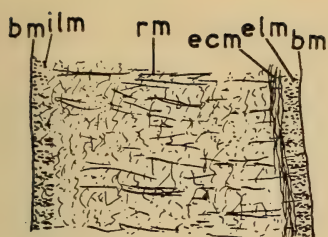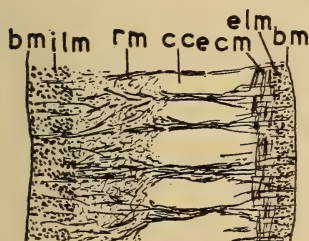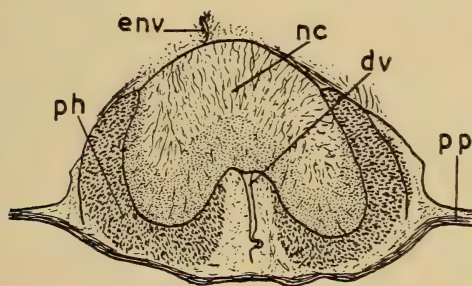
FIG. 60.—*Balanoglossus hydrocephalus* n. sp.   Part of a cross-section through the anterior region of the collar.   × 50.

FIGS. 61–63.—*Balanoglossus studiosorum* n. sp.   61. Part of a cross-section through the anterior region of the collar.   × 50.   62. Cross-section of the collar nerve cord and surrounding structures.   × 36.   63. Median section of the proboscis and collar.   × 3.

bc, buccal cavity. *bm*, basal membrane of the epidermis. *cc*, collar coelomic cavity. *cts*, collar-trunk-septum. *dv*, dorsal vessel. *ecm*, external circular musculature. *elm*, external longitudinal musculature. *env*, epineural vessel. *g*, branchial basket pulled high up into the collar. *ilm*, internal longitudinal musculature. *nc*, nerve cord. *p*, pericardium. *pc*, proboscis coelom. *ph*, perihaemal cavity. *pp*, peripharyngeal cavity. *rm*, radial muscle fibres. *sk*, skeleton. *st*, stomochord.

(fig. 54).   They extend to the dorsal mid-line along the ventral side of the perihaemal cavities, and here a distinct limiting membrane can be seen between the peripharyngeal and perihaemal cavities.   In *B. studiosorum*, on the other hand, the peripharyngeal cavities have fused with the perihaemal cavities (fig. 62).   The circular musculature of the former can be seen running along the ventral wall of the latter. In one of the two sectioned specimens the peripharyngeal cavities widen out along the lateral sides of the perihaemal cavities, and some longitudinal muscle fibres are found here in the peripharyngeal cavities.   A distinct limiting membrane separates the cavities here, but this membrane does not reach the ventral wall.

The collar pores of both species have the usual appearance found in the Ptychoderidae, so that their wall is not very thick and the nuclei in the cells form approximately a single row.   The dorsal fold of the pore is apparent in both species, only in *B. hydrocephalus* this fold is much deeper than in *B. studiosorum*.

An anterior epidermic pouch, dorsal to the place of the anterior neuropore, is quite distinct, although shallow, in *B. hydrocephalus* (fig. 55), but in *B. studiosorum* there is no trace of such a pouch.

As in nearly all species of *Balanoglossus* a central canal in the collar nerve cord is absent, but there are medullary cavities, and these are wider and more numerous in *B. hydrocephalus* than in *B. studiosorum*.   Also at the anterior end of the cord there is no trace of a canal, and therefore also the anterior neuropore is absent. A central canal with a posterior neuropore is present in both sectioned specimens of *B. studiosorum* and in one of *B. hydrocephalus*; in the other specimen of the latter species the nerve cord is solid up to its posterior end.   This canal is present before there is any indication of the splitting up of the collar nerve cord into the dorsal nerve of the trunk and the circular nerves, therefore it cannot be considered to be a simple epidermic depression.   In the two specimens of *B. hydrocephalus* there are 3 and 4 dorsal nerve roots, all of which are rather thin.   The fourth root of the second specimen is very far back near the caudal end of the collar.   In one specimen of *B. studiosorum* I find 2 nerve roots, the anterior of which is very thick and has a wide central canal that ends blindly at both sides. In the other specimen there are 3 nerve roots, the first and last of which also have a similar central canal.   The first nerve root is situated far anteriorly, at the anterior end of the nerve cord.

In a species like *B. hydrocephalus* with a short collar, the anterior surface of which is funnel-shaped, there is no doubt about the position

of the mouth opening. It is found at the bottom of the funnel, where the proboscis neck is fixed to the collar or more exactly where the stomochord opens into the buccal cavity. Here the epidermis of the anterior surface of the collar is continued into the wall of the buccal cavity, and there is a sudden change in the character of the epithelium. Whereas the epidermis is very thin and the nuclei of the cells are scattered over the whole thickness of the epithelium, the wall of the buccal cavity is very thick and the nuclei are found in a thin layer near the surface; a few very small glandular cells, stained very darkly by haematoxylin, are found near the surface (fig. 54). In *B. studiosorum*, on the other hand, the proboscis is connected with the collar about in the middle of the latter, and the part of the collar in front of this connection is not funnel-shaped but cylindrical (fig. 63). In general form, therefore, there is hardly any difference between the part of the collar in front of and behind the connection, and it would at first sight be possible to maintain that the mouth opening is situated at the anterior end of the collar. But in *B. studiosorum* there is a sudden change in the character of the epithelium at the level of the opening of the stomochord, and this clearly indicates the position of the mouth opening. Not only is the wall of the buccal cavity again much thicker than the epidermis at the inside of the anterior end of the cylinder, but the nerve-fibre layer, which as usual is rather thick in the epidermis of the collar, suddenly becomes very thin just where the buccal epithelium begins.

Trunk.—In many Enteropneusta, e.g. *B. stephensoni*, there is a more or less deep groove along the ventral and dorsal mid-line in the branchial region, and the dorsal and ventral nerve cords are found under these grooves. In *B. hydrocephalus* there is such a groove in the dorsal mid-line, but the nerve cord, with the cell layer covering it, is at least as thick as the epidermis alongside of it (fig. 64). In the ventral mid-line there is not even a groove at all, and the nerve cord protrudes. On the other hand, the ventral nerve cord of *B. studiosorum* is found in a median groove and, together with the covering cell layer, is much thinner than the adjacent epidermis (fig. 65). There is also a shallow groove in the dorsal mid-line in this species, but the nerve cord here is not thinner than the epidermis at its sides, and as the glandular cells in the epidermis are swollen considerably, the nerve cord may even protrude here, when the glandular cells are not hypertrophied.

The epidermis of the branchial region contains many glandular cells in both species, some of which are darkly stained by haematoxylin
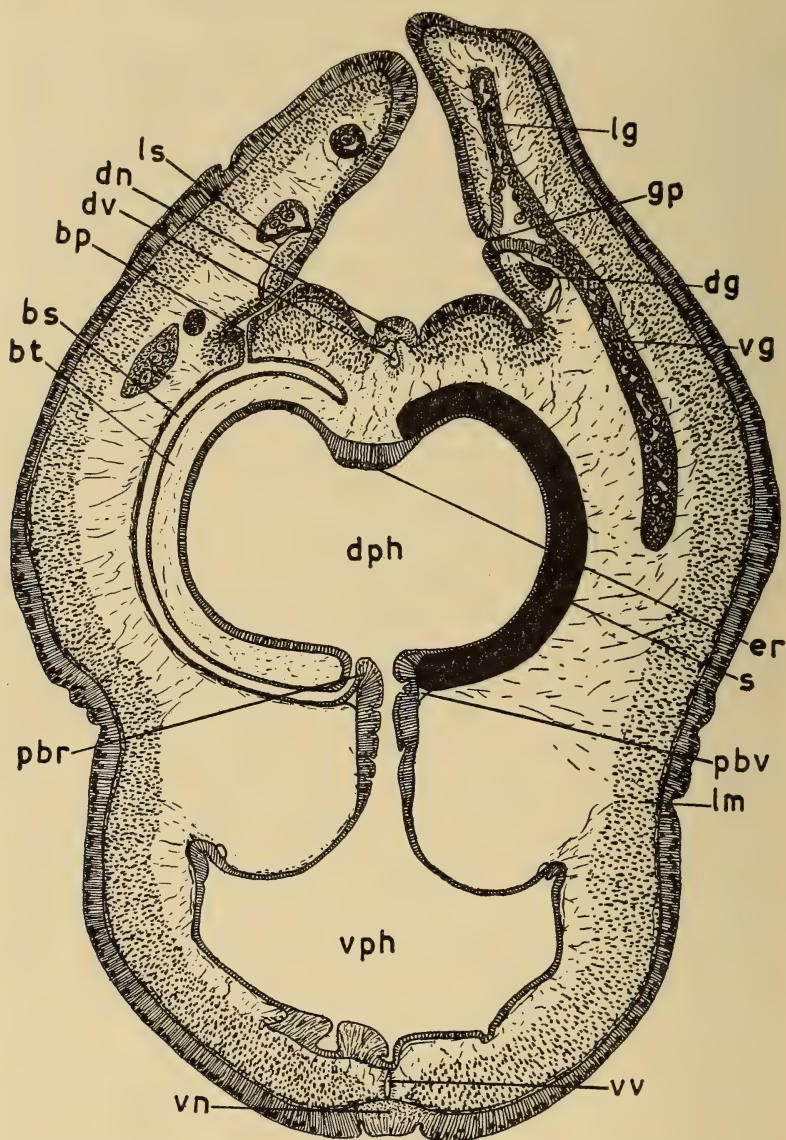
Fig. 64.—*Balanoglossus hydrocephalus* n. sp. Cross-section of the branchial region, partly (branchial basket and gonad at side) composed from a number of sections.   × 12.

bp, branchial pore. *bs*, branchial sac. *bt*, branchial tongue. *dg*, dorsal branch of gonad. *dn*, dorsal nerve cord. *dph*, dorsal pharynx. *dv*, dorsal vessel. *er*, epibranchial ridge. *gp*, genital pore. *lg*, lateral branch of gonad. *lm*, longitudinal musculature. *ls*, lateral septum. *pbr*, parabranchial ridge. *pbv*, parabranchial vessel. *s*, branchial septum. *vg*, ventral branch of gonad. *vn*, ventral nerve cord. *vph*, ventral pharynx. *vv*, ventral vessel.

and are found in the external one- or two-thirds of the epidermis only. In *B. studiosorum* especially these cells form a nearly continuous layer in the epidermis. In this species deep slits occur in the epidermis running at regular intervals from fronto-dorsally to caudo-ventrally
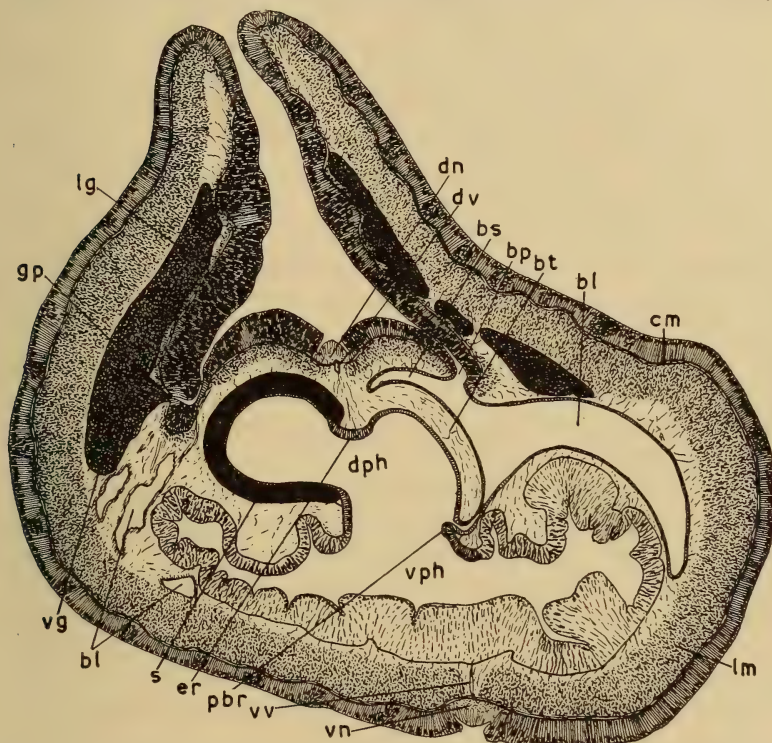

FIG. 65.—*Balanoglossus studiosorum* n. sp. Cross-section of the branchial region, partly (branchial basket, blind-sac at right side, and gonad at left side of figure) composed from a number of sections. × 8.

bl, blind-sac of branchial sac. *bp,* branchial pore. *bs,* branchial sac. *bt,* branchial tongue. *cm,* circular musculature. *dn,* dorsal nerve cord. *dph,* dorsal pharynx. *dv,* dorsal vessel. *er,* epibranchial ridge. *gp,* genital pore. *lg,* lateral gonad. *lm,* longitudinal musculature. *pbr,* parabranchial ridge. *s,* branchial septum. *vg,* ventral branch of gonad. *vn,* ventral nerve cord. *vph,* ventral pharynx. *vv,* ventral vessel.

(figs. 65 and 67). Darkly stained glandular cells are found along these slits, and these reach the basal membrane because the nerve-fibre layer underneath the slits is reduced to a minimum. These slits are so deep that the basal membrane of the epidermis assumes a wavy course. There are also many eosinophil cells in both species, and these are found throughout the whole thickness of the epidermis.

The dorsal side of the body and the inside of the genital wings are even richer in glandular cells than the outside of the wings and the ventral side of the body, and at the dorsal side the cells stained with haematoxylin extend over the whole thickness of the epidermis.

The external circular muscle layer is better developed in *B. studiosorum* than in *B. hydrocephalus*. It is not only slightly thicker in the former species, but it is also continuous and quite distinct along the inside of the genital wings, whereas in *B. hydrocephalus* it becomes so thin that only here and there can a few fibres be distinguished. The longitudinal musculature also is better developed in *B. studiosorum* than in *B. hydrocephalus*, especially on the ventral side of the body and the outside of the wings. On the dorsal side of the body of *B. studiosorum* the layer of longitudinal muscle fibres reaches about half the thickness of that of the ventral side of the body, whereas in *B. hydrocephalus* it is of about equal thickness dorsally and ventrally. As usual, the longitudinal musculature is poorly developed on the inside of the wings in both species. The muscle fibres in *B. studiosorum* are thinner but by far more numerous than in *B. hydrocephalus*.

The dorsal mesentery presumably is absent in the greater part of the branchial region in both species. As, however, the whole branchial region was not sectioned, this cannot be said with certainty, but near the hind end of the branchial region the dorsal mesentery is complete. The dorsal vessel in the branchial region runs along the inside of the epidermis, and the connections between this vessel and the branchial vessels go freely through the coelomic cavity. The ventral mesentery is complete in *B. hydrocephalus*, but in *B. studiosorum* it is not complete throughout; sometimes it is attached to the alimentary canal only, leaving an opening between the right and left coelomic cavities near the epidermis, and sometimes it is attached to the basal membrane of the epidermis only and free from the alimentary canal.

In *B. studiosorum* the ventral, digestive part of the pharynx is wider than the dorsal branchial part (fig. 65). The wall of the ventral pharynx is very thick throughout, and much thicker than the parabranchial or limiting ridges between the dorsal and ventral parts. Therefore the limiting ridges are not very conspicuous in this species. On the other hand, in *B. hydrocephalus* the limiting ridges are well pronounced (fig. 64). In the smaller of the two specimens of this species the dorsal pharynx is about twice the size of the ventral, but in the larger specimen the ventral part has at least the same dimensions

as the dorsal (fig. 64). The wall of the ventral pharynx exhibits a noteworthy peculiarity in *B. hydrocephalus*. According to a figure of *B. clavigerus*, given by Spengel, the dorso-lateral walls of the digestive pharynx are much thinner than the ventro-lateral walls, the whole of this part of the alimentary canal being about quadrangular, and the transition between the thicker and thinner epithelium is very sudden at the lateral edges of the canal. In his description of the species Spengel does not mention this difference in thickness. A similar condition occurs in *B. hydrocephalus*, in which the ventral pharynx is also nearly quadrangular in cross-section. But *B. hydrocephalus* differs from *B. clavigerus* in that the ventro-lateral walls are thin and the dorso-lateral thick (fig. 66). In the ventral mid-line is a groove and here the wall is thicker than the ventro-lateral wall. This difference in thickness was especially clear in the small specimen; in the bigger one the dorso-lateral walls are also rather thin except near the limiting ridge and along the lateral edges (fig. 64). At regular distances a narrow strip of thicker epithelium crosses the thin ventro-lateral wall between the lateral edge and the ventral mid-line. This thicker epithelium does not protrude into the lumen of the pharynx but into the coelomic cavity; it resembles the beams supporting a floor. In the larger specimen it appeared that these strips are in reality deep and very narrow grooves, but as they run in the transverse plane it is difficult to see this in transverse sections.

In both species the branchial pores are too small to be visible externally, therefore it was not possible to count the number of gills directly. But by counting the number of pores in the serial sections it was found that in *B. studiosorum* there occur 32 pores over a length of 4·5 mm. of the branchial region. In the larger specimen of *B. hydrocephalus* 21 pores were counted in a length of 4 mm., but in the smaller specimen the gills are by far more crowded as 21 pores were counted in only 1·65 mm. This cannot be due to contraction only, although it may have been possible that the smaller specimen was more contracted than the larger. Apparently the branchial region extends during growth, not only by an increase in the number of gills at the posterior end of the row, but also by an increase in size of the individual gills, which results in an increase in the distance between the succeeding pores. The estimation of the total number of gills from the numbers given above is rather unreliable, but it is the only method of approach. In the well-preserved and large specimen of *B. hydrocephalus* the branchial region has a total length of 58 mm., and with 21 pores in 4 mm. this would give

a total number of about 300 gills. In *B. studiosorum* it becomes even more difficult, because, as previously mentioned, it is hardly possible to distinguish the posterior end of the branchial region in this species. In a large specimen, in which this could be done, this region was only 22 mm. long, and that would give a total number of about 160 gills.

The epibranchial ridge of *B. hydrocephalus* is made up of a high, convex epithelium (fig. 64). In the middle of the ridge is a number of small glandular cells stained by haematoxylin; in the large specimen this row of glandular cells is broader than in the small one. This central strip with glandular cells is flanked by rows of undifferentiated high epithelial cells, and here the nuclei are situated in a regular row near the free ends of the cells, with some scattered nuclei in the basal half of the epithelium. In *B. studiosorum* the epibranchial ridge is much thinner than in *B. hydrocephalus* and it is hardly convex (fig. 65); glandular cells occur over the whole breadth of the ridge, and all nuclei are scattered.

As usual, the tongue protrudes farther into the pharyngeal cavity than the septum. The septa are narrow, and the epithelium on their back is lower than that on the backs of the tongues; in *B. hydrocephalus* the thickness of this epithelium on the tongues reaches even twice the thickness of that on the septa; in *B. studiosorum* the difference is less. In conformity with the structure of the epibranchial ridge, glandular cells are found over the whole breadth of the back of the tongue in *B. studiosorum*, whereas in *B. hydrocephalus* there are two rows of glandular cells separated from each other by a central row of undifferentiated cells. On the narrow backs of the septa a few glandular cells are also found.

The number of synapticula is the same in both species; it is about 20.

The branchial sacs of *B. hydrocephalus* are rather spacious, and although the wall between these sacs and the coelomic cavity is very much folded, ventral blind-sacs to the branchial sacs are absent (fig. 64). In *B. studiosorum*, as in *B. numeensis* and some other species, these blind-sacs are well developed and reach the ventral wall of the digestive part of the pharynx (fig. 65). Also, at the dorsal side of the branchial sacs blind-sacs are formed that extend into the base of the genital wings, lying there in between or external to the gonads (fig. 68). Small glandular cells occur in the thin walls of the branchial sacs of both species; in *B. hydrocephalus* these glandular cells appear to be more numerous than in *B. studiosorum*. The

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Fig. 66.—*Balanoglossus hydrocephalus* n. sp. Cross-section of the ventral part of pharynx. × 45.
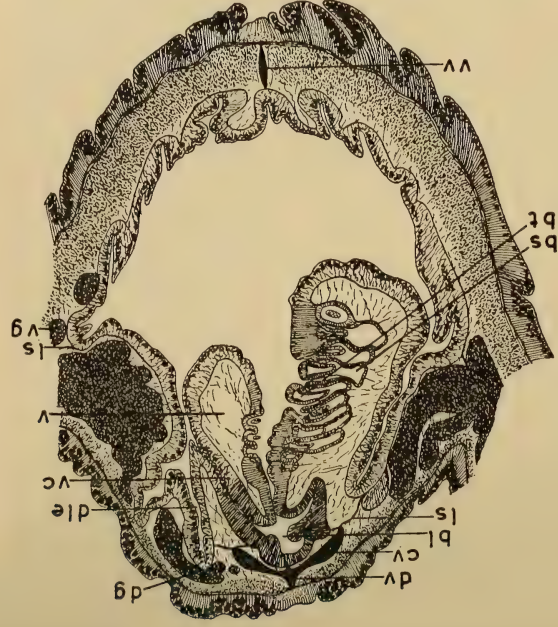
Fig. 67.—*Balanoglossus studiosorum* n. sp. Cross-section at the level of the branchio-genital transition; the genital wings are omitted. × 9.

bl, dorsal blind-sac of postbranchial canal. *bs*, branchial sac. *bt*, branchial tongue. *cv*, commissural vessel. *dg*, dorsal branch of gonad. *dle*, dorso-lateral extension of ventral pharynx. *dv*, dorsal vessel. *gr*, transverse groove. *ls*, lateral septum. *pbr*, parabranchial ridge. *v*, ventral valve. *vc*, ventral connection between the dorso-lateral extension and the dorsal blind-sac. *vg*, ventral branch of gonad. *vph*, ventral pharynx. *vv*, ventral vessel.
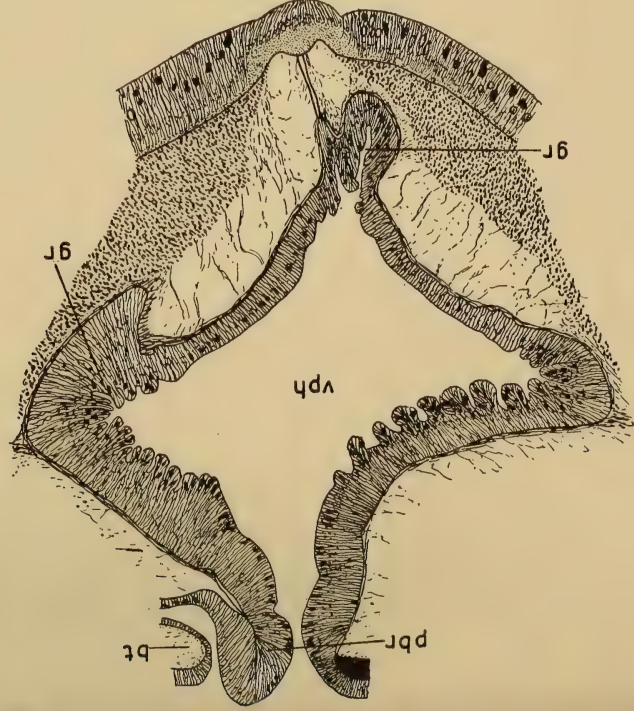
branchial pores of *B. hydrocephalus* are very small, and a short canal, piercing through the longitudinal musculature, connects the branchial sac with the pore.   In *B. studiosorum* such a canal is absent; the sac itself touches the epidermic basal membrane directly, and the pores on the whole are wider than in *B. hydrocephalus*.

In *B. studiosorum* the first two gills, with the collar canal, open to the exterior by a common pore.   The peripheral part of the septum between the first two gills is missing, and in this way these two sacs communicate with each other.   Similarly in *B. misakiensis* the first four gill-sacs are connected and have a common pore.   In *B. hydrocephalus*, as in most species of this genus, the first branchial sacs do not communicate with each other and each sac has its own pore. As in *B. carnosus*, the first pore in both species is situated at the inside of the incipient genital wing, whereas in *B. capensis* and some other species this pore is at the outside of the wing and all the subsequent pores are at the inside.

The postbranchial canal exhibits some features of interest, all the more so as the two species differ from each other in this respect.   In *B. hydrocephalus* the branchial pores are at the bottom of a deep groove, except at the posterior end of the branchial region, where the pores are higher up at the medial side of the groove.   This branchial groove comes to a sudden end; it is even slightly prolonged at its caudal end as a small blind-sac, in which the last gill opens.   Near the posterior end of the row the gills gradually become smaller, as a result of which the epibranchial and parabranchial ridges approach each other and finally fuse behind the last gill-pore.   As the parabranchial ridges are lying close to each other in the branchial region, a narrow median slit-like extension of the wide ventral pharynx is the result of the fusion of the three ridges, much in the same way as described and figured by Maser for *B. clavigerus*.   This narrow dorso-median part of the gut corresponds to the postbranchial canal of other Ptychoderidae.   The wall of this postbranchial canal retains the same structure as the parabranchial ridges and is therefore much thicker than the wall of the ventral pharynx or oesophagus.   In *B. clavigerus* the postbranchial canal has a small blind-sac dorsal to the posterior gills; in other Ptychoderidae, as in most species of *Glossobalanus*, this blind-sac is much larger than in *B. clavigerus*. In *B. hydrocephalus* there is no trace of such a blind-sac.   In the posterior part of the branchial region the lateral edges of the ventral pharynx turn in a dorsal direction and form two narrow dorsal extensions of this part of the gut as is shown in all Ptychoderidae.

These extensions become higher and higher, then their tops turn suddenly in medial direction and fuse with the dorsal end of the post-branchial canal. In this way there is formed on each side a coelomic blind-sac that extends into the oesophagus and that probably acts as a valve in the alimentary canal. The commissural vessels exhibit nothing of particular interest; they are simple transverse connections between the lateral and dorsal blood-vessels, running in the lateral septum and over a short distance, dorsal to the postbranchial canal, free through the coelom.

In *B. studiosorum* the hind end of the branchial region and the postbranchial canal are more complicated than in *B. hydrocephalus.* Further, the deep branchial groove does not gradually become shallower towards the posterior end of the branchial region, but it is suddenly separated from the surface and from there it continues in a caudal direction over a rather long distance in the form of a blind-sac. Quite a number of gills open into this blind-sac. In fig. 67, where some of the last gills are still visible, the blind-sacs have already disappeared; farther frontally they lie between the gills and the dorso-lateral extensions of the ventral pharynx. The postbranchial canal has a well-developed blind-sac, lying dorsal to the posterior gills and extending slightly beyond the caudal end of the branchial region. This blind-sac is clearly defined from the epibranchial and parabranchial ridges, because in the latter small glandular cells are found near the surface of the epithelium, whereas the epithelium of the blind-sac is darkly stained by numerous very narrow glandular cells extending over its whole thickness. The dorso-lateral extensions of the ventral pharynx form ridges increasing in height as in *B. hydrocephalus*; finally, they nearly reach the basal membrane of the epidermis. Already in the posterior end of the branchial region the dorsal blind-sac makes a connection with the two dorso-lateral extensions; this connection is, however, made at about the middle of the height of the extensions, so that a considerable part extends freely farther dorsally. By these connections there is formed a pair of coelomic blind-sacs, in which a part of the posterior gills is still visible (fig. 67). Just behind the branchial region the dorsal ends of the extensions turn to the mid-line and fuse with the top of the dorsal blind-sac, so that another pair of coelomic blind-sacs, lying dorsally to the first, is formed. Both pairs of coelomic blind-sacs protrude backwards into the oesophagus, but as they are small and the oesophagus here is wide, they can hardly act as valves. In *B. hydrocephalus* these valves really block up the whole lumen of the

oesophagus. Each commissural vessel makes two connections with the dorsal blood-vessel.

As only one specimen of each species was cut into serial sections at the level of the postbranchial canal, it is difficult to be certain about the specific value of the form of the postbranchial canal and the valves. *B. hydrocephalus* is almost in complete agreement with *B. clavigerus* in this respect, and there seems to be no doubt that the form of the postbranchial canal, as described above, is characteristic for the species. The two pairs of coelomic blind-sacs, as found in *B. studiosorum*, are not known to exist in any other species. But the animal is quite symmetrical, which makes it at least likely that the two pairs of blind-sacs are a normal feature and of specific value.

The lateral septum of *B. hydrocephalus* extends very far forwards into the branchial region (fig. 64). In most species of *Balanoglossus* the lateral septum extends only over a very short distance into the branchial region; for instance, in *B. capensis* it stops already at the level of the 10th gill-slit from behind. In *B. stephensoni*, with its very short branchial region, the lateral septum reaches about the middle of that region. But in *B. hydrocephalus* this septum extends over nearly the whole length of the branchial region. As previously stated, there may be 300 gills in this species, and I found the anterior end of the dorsal coelomic chamber and therefore of the lateral septum at the level of the 13th branchial pore. How far the septum extends into the branchial region of *B. studiosorum* has not been ascertained. In the two series of sections of the anterior part of the body, which extends a good distance into the branchial region, no lateral septum is present, but it was found over the whole length of a series of the branchio-genital transitional zone.

The row of gonads in *B. hydrocephalus* begins already a short distance behind the collar, the first genital pore being found near the 8th branchial pore. The gonads of the branchial region are very slender, having much the same form as those of *B. clavigerus*; they do not nearly fill the open coelomic space of the genital wings (fig. 64). The lateral lobe of the gonad nearly reaches the edge of the genital wing, therefore the peripheral part of the wing is rigid and not turned in as in *B. stephensoni* and especially in *Glossobalanus marginatus*, as described by Meek. The ventral genital lobe goes down into the body cavity to about half of the height of the branchial basket. A distinct though narrow lumen could be seen in the ovary of the larger specimen, containing many ripe eggs. In the small specimen only a few egg-cells were present, but a great amount of yolk filled

the whole gonads, so that no lumen was visible. In the posterior part of the branchial region the gonads are much larger and fill up the whole coelomic space in the wings. The gonads are also lobed here, whereas more anteriorly they consist of simple lateral and ventral branches. But secondary genital pores, as are found in some other species of *Balanoglossus,* are absent in *B. hydrocephalus.* In most species of *Balanoglossus* dorsal branches of the gonads, lying in the dorsal coelomic chamber, occur only in the genital region and in the most posterior part of the branchial region. But just as the lateral septum extends so far forward in *B. hydrocephalus,* the gonads have dorsal branches nearly from the anterior end of the row (fig. 64). The first dorsal branch, although small, was found at the level of the 25th branchial pore. As the genital pores are rather high up on the inside of the genital wings and consequently the lateral septum is rather broad and the dorsal coelomic chamber rather wide, these dorsal branches of the gonads soon reach a considerable size. The gonads have here three branches, a dorsal, a lateral, and a ventral. In other species of *Balanoglossus* these three branches can be found in the genital region. But in this region only two branches, the dorsal and the lateral, occur in *B. hydrocephalus.* In cross-sections the lateral septum from its insertion to the intestinal wall can be seen going in lateral direction, and then, closely applied to the inside of the external longitudinal musculature, it enters the wing and follows this course up to the level of the genital pore. Here it turns to the inner side of the wing, where it is inserted along the row of genital pores. Therefore branches of the gonads are found only peripherally to the genital pores and in the dorsal coelomic chamber.

The row of gonads begins in *B. studiosorum* at the same level as in *B. hydrocephalus, i.e.* near the 8th or 9th branchial pore. A very small gonad was observed in front of the first genital pore in one specimen, but this gonad does not seem to open to the exterior, at least no pore could be found. The ovaries of the branchial region are much stouter than in *B. hydrocephalus*; there is hardly any room left in the coelomic cavity between the gonads and the longitudinal musculature (fig. 65). But the testes are more slender and repeatedly branched, whereas the ovaries form only blunt lobes. Although the gonads do not quite reach the peripheral end of the genital wings, the top of the wing, nevertheless, is not turned inwards like a loose flap; because of the thickness of the longitudinal muscle layer this part of the wing is rigid. In a ventral direction the gonads reach nearly to the level of the digestive part of the pharynx. But in the frontal end

of the row the ventral branch of the gonad is small and short, as its place is occupied by the blind-sacs of the gills (fig. 65). In the female I find the genital pore much nearer the base of the wing than in the male or than in *B. hydrocephalus*. Secondary genital pores could not be found in the region of the branchio-genital transition. In conformity with most species of *Balanoglossus*, but distinct from *B. hydrocephalus*, the gonads have well-developed ventral branches here; the lateral septum could be followed from its insertion in the intestinal wall peripherally, where it lies between the ventral and dorsal branches of the gonads.

When the testes are quite ripe and full of spermatozoa they exhibit a remarkable form in *B. studiosorum* (figs. 68 and 69). They are profusely branched, and each branch or duct ends in a swollen vesicle filled with spermatozoa. These end vesicles are all turned towards the medial side of the wing, the ducts are along the lateral side applied to the external longitudinal muscle layer along the lateral wall of the genital wing. The yolk cells are well separated from the primary genital cells; the former are found in the ducts and the part of the wall of the end vesicle near the ducts, the rest of the wall of the end vesicles being formed by genital cells. In *B. misakiensis* a similar clear separation between the yolk and the genital cells was found, but here the yolk cells are away from the ducts, and the genital cells form the wall at the side of the ducts.

As I wanted to make out what causes the regular dark rings that make the abdominal region of *B. hydrocephalus* so conspicuous, a part of this region with the caudal region was cut into serial sections. It was found that the epidermis shows here alternating regions that are quite different from each other (fig. 70). In some parts the epidermis is very thin and composed of almost cubical cells. Suddenly these very thin stretches become continuous with parts in which the epidermis is much higher, about five times as high as the thin parts. In these thick regions of the epidermis the nuclei form a dense regular layer in the middle of the cells, and many glandular cells are found in the outer half of the epidermis. The circular musculature under the basal membrane of the epidermis is quite distinct and about as thick as the thin regions of the epidermis; it is continuous and of equal thickness over its whole course. The longitudinal musculature exhibits a remarkable arrangement. It forms a rather thick layer underneath the thick epidermal parts, but it is absent underneath the thin epidermis. It does not even quite reach the edges of the thick epidermal zones. The dark rings, visible externally, are most
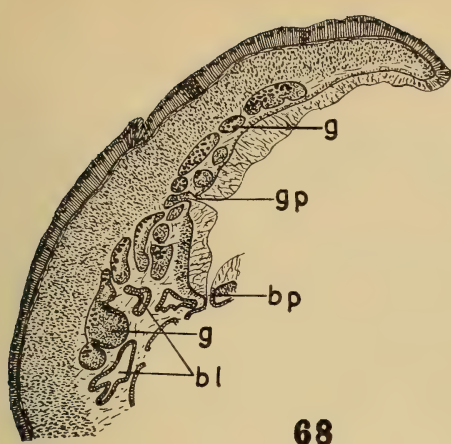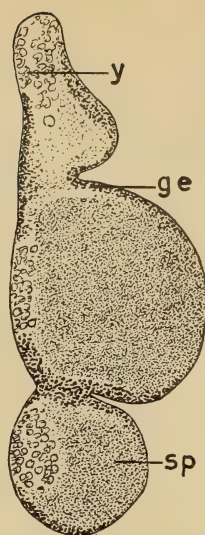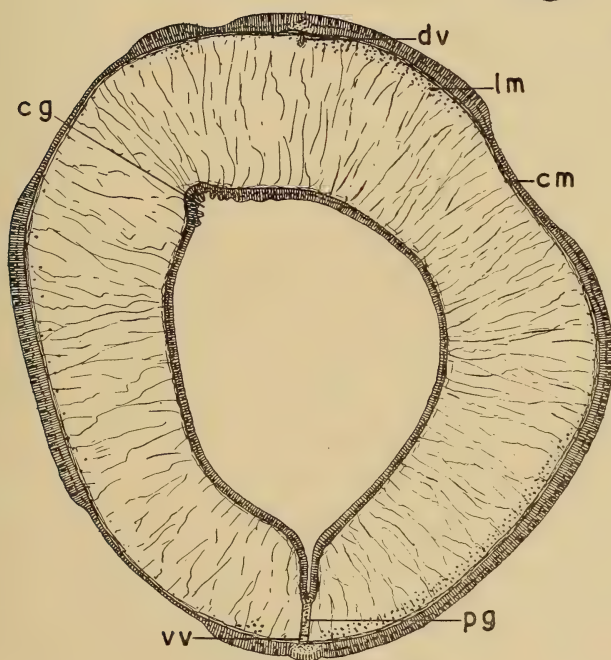
FIGS. 68, 69.—*Balanoglossus studiosorum* n. sp. 68. Cross-section of a genital wing of male. ×10. 69. Part of testis of fig. 68 further enlarged. ×50.

FIG. 70.—*Balanoglossus hydrocephalus* n. sp. Cross-section of the abdominal region. ×30.

bl, blind-sacs of the branchial sacs. *bp*, branchial pore. *cg*, ciliary groove. *cm*, circular musculature. *dv*, dorsal vessel. *g*, testis. *ge*, germinal epithelium. *gp*, genital pore. *lm*, longitudinal musculature. *pg*, pygochord. *sp*, spermatozoa. *vv*, ventral vessel. *y*, yolk cells.

likely formed by the thick epidermis with its many glandular cells; therefore the longitudinal musculature is broken up into rings, which are separated from each other by stretches without these muscle fibres.

A dorsal mesentery is entirely absent in the caudal end of the animal, and the place of the ventral mesentery is occupied by the pygochord. So the latter structure is not confined to the caudal region, but is also found over some length in the abdominal region. It is not separated from the intestinal wall by a limiting membrane, and it exhibits much the same form as that described by Dakin in *Ptychodera flava* (*Pt. pelsarti*) but differs in that it is not swollen ventrally. The pygochord does not show any interruptions, and it is only absent at the extreme caudal end of the animal.

A ciliated groove is conspicuous at one side of the intestine only, occurring for some distance on the right and for some distance on the left side. On the other side it is only indicated by a thickening of the intestinal wall, where the free surface of the epithelium is undulated. This thicker undulating epithelium extends into the caudal region, and finally near the anus it forms the whole wall of the intestine.

Diagnosis of Balanoglossus hydrocephalus.

Total length about 30 cm. Proboscis conical, long, up to 20 mm., collar less than half the length of the proboscis, and at most as long as it is broad; branchial region and genital region of about equal length, 30–40 mm.; liver region long, as long as or longer than branchial region. Posterior end of branchial basket well indicated. Genital wings fused with posterior rim of collar, wings narrow at their anterior end, so that their free edges are separated by a short distance from each other; they reach their maximum breadth near the hind end of the branchial region, and decrease gradually from there; they end abruptly at the beginning of the liver region; no transitional zone present. The liver region is well delimited anteriorly; the saccules in two regular rows, each saccule extends over the whole breadth of the row. At the anterior end the liver saccules soon reach their maximum size and in the first half of the region they hardly decrease in size; in the second half they gradually become smaller, but they are closely applied to each other; it is often hard to determine the hind end of the liver region; there may be about 200 liver saccules. Abdominal region with regular dark rings.

Proboscis.—Circular muscle of equal thickness as nerve-fibre layer;

it does not form a sphincter at the base of the proboscis. Longitudinal musculature strongly developed, fibres more numerous in peripheral than in central part of proboscis. Dorso-ventral muscle-plate well developed, but not extending in front of the central proboscis organs. Large open proboscis cavity in posterior two-thirds of proboscis. Ventral septum extends to end of coelomic blind-sacs. Only left dorsal coelom opens by proboscis pore. No central lumen in body of stomochord; large ventro-lateral blind-pouches not connected with each other; no dorso-lateral blind-pouches. Glomerulus large, extending in front of stomochord and caudally to level of ventro-lateral blind-pouches; the two halves separated from each other by the dorso-ventral muscle-plate; dorsal glomerulus present. Skeleton well developed, with long and prominent keel and thick end-plate.

Collar.—External longitudinal and circular musculature of about equal thickness. Longitudinal musculature along anterior surface of collar thin, and circular muscles here absent. Radial muscle fibres thin and not very numerous. Coelomic cavity almost entirely filled with connective tissue. Dorsal mesentery starts at first dorsal nerve root; from there it may be complete or not to the end of the collar. Ventral mesentery not complete, of varying length. Perihaemal cavities reaching to proboscis pore, almost entirely separated from each other. Peripharyngeal cavities separated from perihaemal cavities. Collar pores with thin epithelium and deep dorsal fold. Nerve cord with anterior epidermic pouch, no anterior neuropore, posterior neuropore may be present, with numerous medullary cavities and 3 or 4 dorsal nerve roots.

Trunk.—No grooves in dorsal and ventral mid-line of branchial region. External circular muscle layer thin, hardly present on inside of genital wings. Longitudinal musculature on dorsal side of body of about equal thickness as that of the ventral side; muscle fibres thick and not very numerous. Dorsal mesentery absent in anterior part of branchial region, present in posterior part; ventral mesentery complete. Parabranchial ridges well pronounced; ventral pharynx smaller than or equal to dorsal pharynx; ventral pharynx with thin wall and thick cross-bars formed by deep grooves along ventro-lateral sides. Number of gills probably about 300; 20 synapticula. Epibranchial ridge high and convex with a strip of glandular cells in the middle. Tongues more protruding than septa; no ventral blind-sacs to the branchial sacs; branchial pores very small; first branchial sacs open to the exterior independently of each other. Postbranchial canal without dorsal blind-sac. Lateral septum extends nearly over

whole branchial region, begins near 13th branchial pore.   First genital
pore near 8th branchial pore; lateral lobe of gonads nearly reaches
the edge of the genital wing; no secondary genital pores; in the
branchial region the gonads have dorsal, ventral, and lateral branches;
no lateral branches in genital region.

Diagnosis of Balanoglossus studiosorum.

Total length about 30 cm.   Proboscis short, extending a few mm.
only from the collar; collar long, up to 20 mm., and about twice as
long as broad; branchial region short, 22 mm., but hind end of
branchial region hardly distinguishable; whole branchio-genital
region about 150 mm. long; liver region short, 10–20 mm. long.
Genital wings fused with posterior rim of collar; they are here already
so broad that their free edges touch each other; they increase rapidly
in breadth, keep the same breadth over the greatest length, and become
narrower only near their hind end; wings end abruptly just at begin-
ning of liver region; no transitional region present.   Liver saccules
of irregular form; anterior end of liver region well defined; posteriorly
the broad row of saccules ends suddenly but may be followed by a
row of small, regularly arranged saccules that gradually become
smaller.

Proboscis.—Circular musculature very thin, about one-third of nerve-
fibre layer; it does not form a sphincter at the base of the proboscis.
Longitudinal musculature poorly developed, concentrated along
central core of connective tissue.   Dorso-ventral muscle-plate almost
entirely absent.   Proboscis cavity greatly reduced, only in ventral
blind-sacs and near proboscis pore.   Ventral septum short and not
reaching the end of the ventral blind-sacs; the latter large and
extending beyond the end-plate of the skeleton; only the left dorsal
coelom opens by proboscis pore.   No central lumen in body of stomo-
chord; ventral blind-pouches small, not connected with each other;
dorso-lateral blind-pouches may be present; neck short and of
irregular form.   Glomerulus small; no dorsal glomerulus.   Skeleton
poorly developed; end-plate thin; body absent or very small; keel
short but prominent; crura broad, thin, anteriorly concave.

Collar.—External longitudinal and circular musculature well
developed and of equal thickness.   Internal longitudinal musculature
very strong, at least as thick as external longitudinal and circular
together in anterior part of collar.   Radial muscle fibres numerous
and thick.   Coelomic cavity rather open; connective tissue only in

central part. Dorsal and ventral mesenteries only present near posterior end of collar. Perihaemal cavities reaching to proboscis pore, almost completely separated from each other. Peripharyngeal cavities not separated from perihaemal cavities. Collar pores with thin epithelium and small dorsal fold. Nerve cord without anterior epidermic pouch; without anterior but with posterior neuropore; with few medullary cavities and 2–3 dorsal nerve roots.

Trunk.—A groove in ventral mid-line of branchial region present, but hardly in dorsal mid-line. External circular muscle layer well developed also on the inside of the wings. Longitudinal musculature on the dorsal side of the body of about half the thickness of that of the ventral side; muscle fibres thin and very numerous. Dorsal mesentery absent in anterior part of branchial region, present in posterior part; ventral mesentery not complete everywhere. Para-branchial ridges thin; ventral pharynx wider than dorsal pharynx; ventral pharynx with very thick wall. Number of gills probably about 160; 20 synapticula. Epibranchial ridge thin and rather flat, with glandular cells over whole breadth. Tongues more protruding than septa; branchial sacs with large ventral blind-sacs, extending also dorsally into base of genital wings; branchial pores rather small; first 2 gills with common pore. Postbranchial canal with dorsal blind-sac; 2 pairs of valves formed by postbranchial canal. First genital pore near 8th or 9th branchial pore. Testes much branched, ovaries lobed. Lateral branch of gonad does not quite reach the top of the genital wings; no secondary genital pores; gonads with dorsal, lateral, and ventral branches in the genital region.

PTYCHODERA FLAVA Eschscholtz.

Ptychodera flava, ubiquitous in the tropical Indo-Pacific region, occurs also near Inyack Island, and this is the most south-westerly locality in which it has been found up till now. It occurs on a flat, to the south-west of the island, that is dry at low tide. A great number of small specimens were procured here by turning over stones.

LITERATURE.

ABEL, O., Vorzeitliche Lebensspuren. Jena, 1935.

DAKIN, W. J., "A New Species of Enteropneusta, *Ptychodera pelsarti*, from the Abrolhos Island," Journ. Linn. Soc. Zool., vol. xxxiii, 1916.

GILCHRIST, J. D. F., "On Two New Species of *Ptychodera* (*P. proliferans* and *P. natalensis*)," Ann. S. Afr. Mus., vol. vi, 1908.

HILL, J. P., "On a New Species of Enteropneusta (*Ptychodera australiensis*) from the Coast of New South Wales," Proc. Linn. Soc. N.S.W., 2nd ser., vol. x, 1894.

——, "The Enteropneusta of Funafuti," Mem. Australian Mus., vol. iii, 1897.

HORST, C. J. VAN DER, "West-Indische Enteropneusten," Bijdragen tot de Dierkunde, vol. xxiii, 1924.

——, "Quelques observations anatomiques sur l'entéropneust *Glandiceps talaboti* (Marion)," Bull. Soc. Sc. Nat. du Maroc., vol. ix, 1929.

——, "Observations on Some Enteropneusta," Vidensk. Medd. fra Dansk naturh. Foren., vol. lxxxvii, 1930.

——, "The Burrow of an Enteropneust," Nature, vol. cxxxiv, 1934.

——, "On a New South African Species of *Balanoglossus* and a Comparison between it and *Balanoglossus capensis* (Gilchrist)," Ann. S. Afr. Mus., vol. xxxii, 1937.

KAPELUS, F. FROOTKO, "The Anatomy of the Enteropneust *Saccoglossus inhacensis* sp. n.," Ann. Natal Mus., vol. viii, 1936.

KOWALEVSKY, A., "Anatomie des Balanoglossus Delle Chiaje," Mém. de l'Acad. Imp. des Sciences de St.-Pétersbourg, VIIe ser., vol. x, 1866.

MASER, O., "Über *Balanoglossus carnosus* (Willey) und eine ihm nahestehende neue Art von Neucaledonien," Zool. Jahrb. Abt. Anat., vol. xxxiii, 1913.

MEEK, A., "*Glossobalanus marginatus*: a New Species of Enteropneusta from the North Sea," Quart. Journ. Micr. Sc., vol. lxvi, 1922.

PUNNETT, R. C., "The Enteropneusta," The Fauna and Geography of the Maldive and Laccadive Archipelagoes, vol. ii, pt. 2. Cambridge, 1903.

SPENGEL, J. W., "Die Enteropneusten des Golfes von Neapel," Fauna und Flora des Golfes von Neapel, Monogr. 18, 1893.

——, "Eine neue Enteropneustenart aus dem Golf von Neapel, nebst Beobachtungen über den postbranchialen Darm der Ptychoderiden," Zool. Jahrb. Abth. Anat., vol. xx, 1904.

——, "Studien über die Enteropneusten der Siboga-Expedition nebst Beobachtungen an verwandten Arten," Uitkomsten Siboga-Expeditie, Monogr. 26. Leiden, 1907.

STIASNY, G., "Zur Kenntniss der Lebensweise von *Balanoglossus clavigerus* Delle Chiaje," Zool. Anz., vol. xxxv, 1910.

WILLEY, A., "Enteropneusta from the South Pacific, with Notes on the West Indian Species," Willey's Zoological Results, pt. iii. Cambridge, 1899.